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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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BIOLOGICAL BULLETIN

THE OVIPOSITION, COCOONING AND HATCHING OF AN ARANEAD, THERIDIUM TEPIDARIORUM C. KOCH.[1]

THOS. H. MONTGOMERY, Jr.

During the course of collecting a series of accurately timed stages of the eggs of one of our most familiar spiders, the *Theridium tepidariorum*, I had occasion to accumulate quite a number of observations upon the reproductive habits. Published accounts of such habits in spiders are very few, there is much still unknown, so that it seemed worth while to write up these notes since they are the most extensive yet made upon any single species.

In an earlier contribution[2] I presented an account of this species along with others, and described the moulting, copulation, sperm-induction by the male, cocooning and care of the young. I then described three cases of cocooning, and timed the sequence of cocoons for eight females.

The present observations were made at Woods Hole, Mass., from the fifth to the twenty-sixth of August of the past summer. An unusually large colony of these spiders was found upon an old stone wall in a wood, to which they had probably strayed from some buildings adjacent to one end of the wall. About the first of August the spiders were beginning to form their first cocoons, and upon each of most of the webs of females one or more males were present. Two or three weeks later most of the males had disappeared. My previous study showed that there may be frequent copulations before the first act of oviposition, and even between successive acts. But since the males begin to disappear before the full series of cocoons is made, and especially because my captive females produced successive cocoons without the presence of males, and yet these eggs were fertile, it is apparent that copulation before the first act of oviposition suffices for the fertilization of the later sets of eggs. Nearly 150 females were kept in small cages consisting of wooden and paper boxes covered on one side by glass ; there was very little mortality among them, and at the end of the month they were manumitted in good condition.

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 76.

[2] "Studies on the Habits of Spiders, particularly those of the Mating Period," *Proc. Acad. Nat. Sci. Philadelphia*, 1903.

The processes of oviposition and cocooning were observed in part or completely in a large number of cases, and may be summarized as follows. The female commences the cocooning by biting through certain threads in a particular region of the web ; she then gathers the cut ends together with her legs, thus making a free space in which to work and at the same time forming a composite thread that serves for the initial suspension of the cocoon as well as of herself during the process of construction. She spins upon the lower end of the suspension thread, and combs out the new line into a fluffy ball ; this is the beginning of the base of the cocoon, and the spider employs for the combing mainly the third pair of legs, but to some extent the fourth also. From this point in the operations until the cocoon is nearly completed the spider hangs to lines of the surrounding web by her first and second pairs of legs, with her cephalothorax directed vertically and above the abdomen. When the fluffy fundament of the cocoon base has a diameter somewhat larger than the length of the cephalothorax of the spider, the latter rotates this loose textured ball with her palpi and third pair of legs, and with the fourth pair in alternate application draws a thread out from her spinnerets and applies it to the lower rim of the fluffy ball. She continues this motion until the base of the cocoon has attained the form of an inverted cup, the upper convexity of which is attached to the suspensory line. Towards the close of the base-making her spinning becomes gradually slower. Then she proceeds to oviposit. She lifts herself slightly by contracting the anterior pairs of legs, then presses her genital aperture against the concavity of the base; a yellow drop of fluid pours out and immediately adheres to the concavity of the base, the eggs rapidly flowing out into it until the whole reaches a size quite equal to that of the spider's abdomen. The spider touches the surface of the egg mass only with the lower surface of her abdomen; during the oviposition there are repeated pressures of the abdomen against the egg mass. The yellow fluid is at first of thin consistency and soon dries upon the eggs; but in drying it does not glue them together. Shortly after the discharge of all the ripe eggs the spider by a few quick contractions detaches her epigynum from the surface of the egg mass, and immediately starts to make the cover of the cocoon, spinning first upon the exposed surface of the egg mass until the latter is covered, then upon the surface of the base also. From her spinnerets she draws out a continuous, compound thread with the fourth legs used alternately, applying this thread to the growing cocoon, while at the same time rotating the cocoon slowly with the third pair of legs (with the occasional help of the second), the spider still hanging to the web above the cocoon by the first pair. Thus the eggs become quickly hidden from sight, by the making of a closely knit cover composed of one continuous thread. Throughout the whole operation the spider keeps pressing the tips of her palpi against the cocoon, which gives her a knowledge of the progress of the progress of the work; there can be no question that she is guided entirely by the sense of touch, for during her labor she is is so placed as to be unable to see the cocoon. Towards the close of the cover making she spins closer, with the result that the exterior surface of the cocoon has the firmest texture. The spinning finished, she feels over the entire surface with her palpi. Rarely does she leave the cocoon hung upon the suspensory thread, usually she carries it higher up into the web and attaches it to the object that roofs the latter.

The finished cocoon varies from yellowish to dark brown in color, and large ones may reach a diameter of 10 mm. It is quite smooth externally, pyriform and usually pointed at the upper end where the suspensory thread was first attached.

More than two hundred cocoons were produced by my spiders, of which 188 were marked as to the exact or approximate time of oviposition. On a few days I did not commence observations until 6 a. m., and in such cases estimated the time of oviposition of prior cocoons by the state of completion of the cover; but in the majority of cases the precise time of the end of the act of oviposition was determined. From the accumulated data the following results were obtained:

1. *Time of Day of Oviposition.* — This is probably always in the morning and usually in the early morning; in one case a cocoon was made some time between 11 a. m. and 4 p. m., while I was absent from the laboratory, and in this single case oviposition may have occurred later than the noon hour. The next latest case was one at 11.29 a. m. The following table shows the hours of oviposition and the number of cocoons formed in each:

Before 5	5–6	6–7	7–8	8–9	9–10	10–11	11–12
43	51	42	32	33	9	5	2

In the laboratory the spiders experienced about the same light conditions as they did upon the stone wall. Though my lamp was frequently burning at night in the room, and its light wakened the spiders to activity, yet it never induced them to start cocooning. The influence that occasions the cocooning can therefore not be one of amount of light, nor of sudden change from darkness to light. If it were amount of light they should be expected to cocoon at twilight as well as at dawn, yet they never do so. I do not think that cocooning is stimulated by any light condition, but that the stimulus to oviposition may be the oncoming of the warmth of the day after the coolness of the night. My records of cocoons of one and the same individual show that successive cocoons may be formed at different hours of the day, *i. e.*, that one individual does not maintain a particular cocooning hour.

2. *Time Duration of the Act of Oviposition.* — This was timed in 43 cases, the interval being measured from the moment of commencing extrusion of the eggs to the moment of commencing spinning of the cover. In one case it lasted just 2 minutes; in nine cases from 3 to 4 minutes; in twenty cases, from 4 to 5 minutes; in five cases, from 5 to 6 minutes; in six cases from

6 to 7 minutes; in one case, 8½ minutes and in one case, where the spider seemed feeble, 19 minutes. In the majority of cases it lasts from 3½ to 4½ minutes. There is remarkable uniformity in the length of duration of the act, which seems to be quite independent of the number of eggs discharged. After all the eggs have been extruded but while a point of the egg mass surface still adheres to the genital aperture, the spider may continue reiterated ineffectual efforts to discharge further eggs, and how long it continues to do so renders the duration of the process longer or shorter.

3. *Time Duration of the Spinning of the Base.* — This was timed for seventeen cases, showing a variation from 14 to 37 minutes; in eleven of these cases it was between 20 and 30 minutes.

4. *Time Duration of the Spinning of the Cover.* — This was observed in thirty-two cases. In one case the process lasted 27 minutes; in five cases, 40 to 49 minutes; in eleven cases, 50 to 59 minutes; in two cases, 60 to 69 minutes; in three cases, 70 to 79 minutes; in four cases, 80 to 89 minutes; in three cases, 90 to 99 minutes; in one case, 110 minutes; and in one case where the spider lacked the left fourth leg, for 142 minutes. There is, accordingly, considerable variation in the duration of this operation, due not at all to the size of the egg mass, apparently also not to the rate of spinning, but rather to the thickness and firmness of the cocoon which varies greatly. The cover making is usually uninterrupted, but sometimes the spider may pause, then evidently from weariness. It may be that high nourishment would allow the greatest amount of silk secretion, and that in well fed individuals the cocoons be largest and take the most time in the making.

5. *Abnormalities in Cocooning.* — Abnormal cocoons are not rare with wild individuals, and relatively more numerous with my captive specimens. Generally an abnormal cocoon is due to a defect in the spinning of the base; the latter may be too small or too loose, and the spider in working upon it is then liable to pull it out into an irregular form. Again, the issuing egg mass may by accident adhere to a leg of the spider, or even a portion of it become detached from the rest; when this happens the spider

may cease to spin, seemingly being cognizant of something out of the ordinary course. Or the cover may be incompletely made, leaving the egg mass partially exposed, this is apparently due to lack of sufficient space for the spinning operations. Once a mistake is made the spider appears to be unable to rectify it, a fact that I have remarked in my earlier observations (*l. c.*); the whole process appears to be strictly instinctive and the spider seems unable to modify it more than quantitatively, and unable to learn by experience. An individual may make an imperfect cocoon, then construct a following one quite perfect; or the reverse may happen. When the cocoon is particularly irregular, as when the egg mass becomes more or less broken, the spider may either eat the eggs, or may detach them from the web and allow them to fall to the ground.

6. *Time Intervals between Successive Cocoons Formed by the same Individual.* — Of 113 timed intervals between successive cocoons raised in my cages, there was one interval of 2 days, 2 of 3 days, 13 of 4 days, 26 of 5 days, 18 of 6 days, 19 of 7 days, 12 of 8 days, 10 of 9 days, 3 of 10 days, 4 of 11 days, 4 of 12 days, and 1 of 13 days. More than half of the intervals ranged from 5 to 7 days. In all probability the rapidity in the rate of the succession of cocoons depends upon the degree of nourishment of the spider, because some that I purposely starved furnished no cocoons at all. My captives were of course not as well fed as they would have been in the wild state, and though I kept them fairly well supplied with young grasshoppers and locustids this by no means equalled their natural diet in either variety or amount. It is probable that these spiders in a natural state, with normal feeding, would furnish on the average cocoons at intervals of from four to six days. The shortest time in which a succession of 4 cocoons was made by any one of my spiders was 14 days, comprising one interval of 4 days and two of 5 days. The largest number of cocoons I have found in any wild web was eight.

7. *Hatching of the Young.* — Thirty-nine cocoons were kept to determine the time of hatching of the young. Of these, 3 failed to hatch; 3 hatched after an interval of 11 days; 3, of 13 days; 18, of 14 days; 2, of 15 days; 9, of 16 days; and 1, of

17 days. The majority, accordingly, hatched at intervals of from 14 to 16 days. Different generations of cocoons do not have different rates of hatching. Cocoons made on the same day need not hatch at the same time; thus of 8 cocoons made on August 20, 3 hatched in 11 days, 2 in 13 days, 2 in 14 days, 1 in 16 days; and of 11 cocoons made on the morning of August 26, 9 hatched in 14 days, and 2 in 16 days. All these cocoons were removed from the web, placed in separate glass vials, and kept together under the same conditions of light, temperature and moisture. The differences in the rate of hatching are probably due to the difficulties experienced by the young in emerging, for where the cocoon is thinnest the hatching is earliest. Most of the spiderlings emerge in the early morning, but they may come out in the late morning and the afternoon. Each of the few most vigorous spiderlings makes a small circular aperture through the wall of the cocoon, and through these few openings all the rest find their way. The young are at first decidedly positively heliotropic, and it is the light shining into the first made exits that probably guides the less precocious individuals out of the cocoon. The weakest may not emerge until several hours or even days after the most vigorous. In this species, unlike the lycosids, I have found no evidence that the mother aids the young to hatch; for I removed from the webs a number of cocoons immediately after their completion, thus precluding any opening of the cocoon by the mother, yet all of the young made their way out. The mother exhibits some degree of bravery in guarding the cocoon, especially when the latter is newly formed, though never to the extent of allowing herself to be injured, and if she is roughly handled she invariably drops from the web.

8. *Protective Value of the Cocoon.*—I removed eggs, at various intervals after oviposition, from cocoons and placed them in flat glass dishes in the ordinary diffuse light of the laboratory; in all these cases normal spiderlings resulted. Therefore the presence of a cocoon is not necessary to normal development and, further, its value is probably not to exclude the light. One batch of eggs was opened onto a dish of water, where they floated; after a number of days they become covered by a mould that killed

them. Probably the main value of the cocoon is to protect the eggs from enemies, for were the eggs not enclosed they would fall singly or in a mass to the ground, become removed from the guardianship of the mother, and be subjected to a great variety of rapacious foes.

9. *Relation of the Cocoon to the other Forms of Aranead Architecture.* — On this important question I will dwell only briefly at this point, reserving a fuller discussion for later. McCook[1] concludes: "The spinning work of spiders may be classified generally as, first, the Snare, spun for the capture of prey; second, the Enswathment, by which insects are disarmed and prepared for food; third, the Gossamer, used for purposes of aqueous and aerial locomotion; fourth, the Cocoon, spun for the propagation and protection of the species; and fifth, the Nest, which is a domicile more or less elaborate and permanent within and under which the aranead dwells for protection against the exigencies of weather and the assaults of enemies." Menge[2] had previously called attention to the web that the males of certain species spin for the deposition of their sperm, preparatory to charging their palpi with it; this has also been described by me (*l. c.*), and it may be called the "sperm-web." Then Wagner[3] in a most important analysis of the architecture of spiders directed especially towards its phylogenetic significance, has distinguished the web spun by some forms for protection during the moult; and then divides what McCook calls the "Nest" into: "*La retraite*-construction, destinée pour *la demeure de l'araignée*, où elle passe tout son temps et qu'elle ne quitte que pour la chasse. A l'epoque de la ponte cette retraite peut servir de loge au cocon." And into "*Le nid*-construction, destinée au séjour de la femelle et à contenir le cocon." As far as I comprehend this distinction of Wagner's, the "retraite" is a nest made by both sexes, the "nid" one built by the female only. It seems to me questionable whether this distinction of Wagner's is a valid one, and McCook's term "nest" had best be retained without especial subdivision, though surely different kinds of nests may be distinguished.

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[1] "American Spiders and their Spinning Work," Vol. 1, Philadelphia, 1889.

[2] "Ueber die Lebensweise der Arachniden," *Neueste Schr. naturf. Ges. Danzig*, 4, 1843.

[3] "L'Industrie des Araneina," *Mém. Acad. Sci. St. Pétersbourg* (VII.), T. 42.

Therefore we have to enumerate: the *cocoon*, *snare*, *nest*, *parachute* (a somewhat more definite term to replace McCook's "gossamer"), *sperm-web*, *enswathment* and *moult-web*. Of these constructions the cocoon occurs in all species, and probably the nest also; these may then be said to be the most conservative or most persistent kinds of architecture in spiders. It is probable that the nest or protective habitation was the first construction to be elaborated, perhaps first merely some cranny that later became lined with silk by the spider; and that the spider laid its eggs first without lining against the wall of this nest, then later spun upon them a cover of silk. This is of course merely an inference because we know nothing of the ancestors of the modern spiders. But the cocoon, by which is meant the immediate silken envelope of the eggs, may in this way be explained as a derivation of the nest of which it formed at first an integral part; the original base of the cocoon would have been the silken investure of the nest, the cover of the cocoon a later addition. If this suggestion be correct, then the phylogeny of the cocooning would have been as follows: (1) oviposition against the wall of the nest; (2) the addition of a cover to the eggs; (3) a special base spun upon the wall of the nest, the eggs upon which received a cover (a condition realized by certain attids, clubionids and drassids); (4) the formation of a cocoon with base and cover apart from the nest.

Wagner has distinguished two kinds of cocoons: those made of two pieces, and those of a single piece, a distinction based upon the appearance of the completed structure. I would maintain, on the contrary, that it is probable that in all modern araneads the cocoon is spun of two pieces, first a base, then a cover, for I have observed (*l. c.*) this process in lycosids, agalenids, dictynids, theridiids, epeirids and drassids. One may not judge from the form of the finished cocoon as to its mode of construction, and, so far as I know, in all cases where the process has been followed a base is first spun, the eggs oviposited upon it, then a cover made. There seems to be no positive evidence that the cocoon is ever made in a single piece. One of the best diagnostic characters of Araneads is the making by the mother of a silken covering, composed of two pieces, for the protective investment of the eggs.

10. *Parasitic Hymenoptera in the Eggs of Theridium.* — In none of the cocoons raised by my captive spiders were there any parasites, but about one out of every thirty or forty wild cocoons was found to be infected with a tiny hymenopteron, black in color, the males winged and the females wingless, of a length somewhat less than the diameter of an egg of the spider. Prof. Wm. H. Ashmead has kindly identified these as Proctrotrypids, constituting a new species (*montgomeryi*) of the genus *Bæus*. The males hatch some hours or a day in advance of the females, and die soon after the momentary copulation with the latter. The wasps generally hatch out from the cocoons at about the same time the spiders hatch from the uninfected eggs.

Three cocoons, from four to six hours old, were placed in a dish containing wasps that had hatched from other cocoons one or two days previously. The female wasps walked over the surface of the cocoons, continually tapping them with their antennæ; most of the females oviposited into the cover of the cocoons, or at least frequently projected their ovipositors into them. A smaller number made their way into the interior of the cocoons, taking about ten minutes to bore, mainly by rotation of the large and sharp-rimmed head, a small circular aperture into the cocoon. After several of the female wasps had entered in this way I cautiously opened a cocoon and under the compound microscope could observe the wasps piercing the spider eggs with their long, needle-like ovipositors; they seem not to succeed in piercing every egg that they attempt. A single wasp inoculates in this way a considerable number of spider eggs. Only one wasp emerges from any one spider egg, so it is probable that the wasp places only one egg in a spider egg, but I hope to decide this point by a careful study of the infected eggs. Rarely if ever are all the eggs in a cocoon so infected; the infected ones after three or four days turn a dull brown color. If an infected cocoon is kept until hatching in a closed vial, so that neither the emerging spiders nor the hatching wasps can escape, the spiderlings that have escaped infection ultimately catch and eat the adult wasps.

REGENERATION OF CERTAIN STRUCTURES IN FUNDULUS HETEROCLITUS.

C. V. MORRILL, Jr.

It is a fact of common knowledge to all observers of fish kept in aquaria, that portions of the fins, lost through accident, or purposely removed will be replaced by new structures which cannot be distinguished, in many cases, from normal fins. This ability to regenerate has been examined by Duncker (1905), Morgan (1900, 1902), Nussbaum and Sidoriak (1900), Mazza (1890) and Broussonet (1786). The following experiments were made to test the effect of cutting off the fins close to the body. The regenerative powers of the operculum, the lower jaw, the scales and the lens were also tested.

The various sets of experiments described below were tried on different fish, no one fish having more than one fin removed, except in the case of pectorals and pelvics which were removed from the same fish, the right pectoral and left pelvic being always selected for convenience.

I wish to express my thanks to Prof. T. H. Morgan, who kindly suggested this problem and under whose direction it was completed. My thanks are also due to the staff of the New York Aquarium, and to Dr. F. B. Sumner, director of the U. S. Fisheries Laboratory at Wood's Hole, Mass., who kindly placed the necessary tanks and fish at my disposal.

Dorsal Fin.

On October 10, 1905, eighteen fish had the dorsal fin cut off as close to the body as possible. These were examined from time to time until December 29, 1905, when due to various causes, principally fungal growth, the number had become reduced to thirteen. All of these with one exception were regenerating a new fin. The excepted case was kept under observation three months longer but at the end of that time, twenty-six weeks after operation, it had developed only a small pointed knob in the middle of the old scar. The remaining twelve were not kept after December 29 (twelve weeks after operation). In all of these the regeneration was proceeding rapidly at this time, some having the new fin half completed.

On February 16, 1906, a second set of twenty-five fish had the dorsal fin cut off close to the body. These regenerated more rapidly than the first set and in five weeks' time, twenty-one of these which still remained, were regenerating along the entire length of the cut surface, in many cases the new growth being one eighth of an inch in height. Three weeks later, fifteen only were still living; the increase in height of the fins was distinctly visible in this short time, though more so in some individuals than in others. Three weeks later (at the time of writing) and twenty-three weeks after operation, fourteen were still alive. The new fins in some cases were regenerating evenly and possessed the normal number of fin rays (eleven). But in other cases, the new growth appeared along part of the cut surface only. An examination of the anatomy of the fin and the exact direction of the cut reveals the cause of this irregularity. Usually in trimming the fin close to the body, the dermal fin-rays are severed a short distance distal to their articulation with the bony supporting rays, beneath the surface. Sometimes, however, if the flesh at the sides of the fin is pressed down by the scissors and the fin at the same time held firmly, the cut will pass, in some places, through the enlarged proximal part of the dermal fin rays and regeneration in consequence will be much slower, or may not take place at all in parts so affected. The cut may even at times remove the dermal rays completely. These facts, I think, will account for cases in which regeneration is irregular, *i. e.*, when the new structure does not contain the normal number of fin-rays. Thus complete or nearly complete removal of the fin-rays prevents regeneration of the fin.

Anal Fin.

The anal fins of eighteen fish were cut off close to the body on October 25, 1905. The wounds healed up rather quickly but showed no signs of regeneration until two months later, when of the thirteen fish remaining, five showed slight signs of new growth. Six weeks later the regeneration was fairly started in five fish which still survived. These five were examined one week later, *i. e.*, sixteen weeks after removal of the fin and it was found that three showed slight regeneration while the other two had a new fin about half the size of the original one. In these last, the fin was normal in shape and position, though small, and the rays parallel to one another as in the old fin. Ten weeks later, twenty-one weeks after removal of original fin, the regeneration was almost complete, the new fin having the normal number of fin-rays and being scarcely distinguishable from ordinary anal fins.

Here as in the case of the dorsal fin, the cut usually passes a short distance distal to the articulation of the dermal with the bony fin rays. But it may pass a little further in through the articulations if not carefully made, as the body is soft in the region of this fin. A cut of this kind results, as might be expected, in no regeneration. The cut may, however, pass through the articulations of some rays and the shafts of others ; then the new growth takes place from the cut ends of the latter only, producing an irregular structure with less than the normal number of fin-rays.

The external prolongation of the oviduct in the females, which passes down the anterior end of the fin is, of course, severed with the fin. It regenerates, as might be expected, only when the anterior fin-ray which supports it, regenerates, not otherwise.

CAUDAL FIN.

On October 5, 1905, the caudal fins were removed from eighteen fish, the cut being made in a curve so as to remove the entire fin, without at the same time cutting off any of the tail vertebræ proper. This operation is rather severe ; there is usually some bleeding and the edges do not close over the wound very readily. In consequence the number of fish lost is usually considerable. After twelve weeks, five fish only remained, all showing regeneration of the fin to some extent. Two weeks later the regeneration was still proceeding slowly but somewhat irregularly, the new fin-rays being bent downward, forming a considerable angle with the original horizontal direction of the rays, and the rays themselves were fewer in number than in normal fins. This result was due to very close cutting; entire rays were removed in some places, and in others, where stumps remained an alteration in direction of these stumps occurred in some way when the wound healed over. In one case the new fin grew straight out in the normal direction but at different rates of growth in different parts so that it presented a finger-like appearance. This abnormality was probably due to a jagged cut, the fin-rays being disarticulated in some places while in others, the stumps were left in situ. In still another fish no regeneration took place at all although the wound healed over smoothly. This fish was kept under observation four months. Here the cut probably either disarticulated the rays or left too small a stump for regrowth to take place.

On January 9, 1906, the tails of twenty-four fish were cut off in such a way as to remove a portion of the body of the fish, including several vertebræ. Obviously this operation was extremely severe. These fish when returned to water were unable to assume a horizontal position but remained head downward at the top of the tank. Three days later eleven were still living. These latter had succeeded finally in reaching the bottom of the tank and some were swimming about in a nearly normal position. At this time, January 12, another set of twenty-four fish, cut as described above, were put into the same tank with the eleven just mentioned. Three days later only seventeen were left of the entire number, thirty-five. This number gradually decreased until the entire lot died within a month's time.

Another set of twenty-four were cut in the same way but it was found impossible to keep them alive. The wounds remained open and the flesh rotted away from the sides of the vertebræ.

In the above sets, various sizes of fish were used. In still another set ten *small* fish were employed and kept in shallow dishes in the laboratory in order that they might assume a horizontal position. Within a month all of these had died with one exception. This might have been due to unfavorable conditions in part, though I have kept fish living in these shallow dishes over three months. In the exception noted, the wound had healed over smoothly and a very small white knob of tissue appeared over the cut end of the spinal cord. This fish was kept under observation for three months but during this time the white knob did not increase perceptibly in size. As far as external examination could determine no regeneration of bone took place.

Pectoral and Pelvic Fins.

On October 25, 1905, twenty-four fish had their right pectoral and left pelvic fins removed. The cuts were made as close to the body as possible. In three months all died but three. These showed no regeneration of the pectoral fin but in one case a slight indication of a regenerating pelvic. Two weeks later two of these were still living, both showing the regenerating pelvics well under way and a well-defined indication of regenerating pectorals. Unfortunately the two fish died a few days later. Another set of twelve fish were cut in the same way but all died within a month, showing no signs of regeneration.

A third set of twenty-five fish cut as above, were started on February 16, 1906. Nine weeks later eighteen were still living; five of these showed regenerating pelvics and one, an indication of regenerating pectoral; the rest no regeneration of either fin. Two weeks later (at the time of writing) seventeen were living. Two of these showed regenerating pectorals distinctly, in the shape of a small knob of tissue which no doubt contained two or three regenerating rays, since the fish moved the structure to and fro like a fin. Eight of this same lot were regenerating their missing pelvic fins, the new structure in some cases being one eighth of an inch long.

An examination of the direction of the cut in the removal of the pectoral fins shows that in most cases all the dermal fin rays are removed entire, *i. e.*, disarticulated. Occasionally, however, the cut does not completely remove the uppermost one or two rays. These rays extend inward a little further than the others and so their proximal stumps may remain in situ and give rise to little pointed structures as described above, but never to a complete fin. In the case of the pelvic fins, it is not possible as a rule to cut them so close in; thus it more frequently happens that stumps of the rays are left, sometimes a few, sometimes all. Regeneration takes place from the cut ends of these only.

From the foregoing facts, the lack of regeneration of the paired fins in so many cases is seen to be due to complete removal of the dermal fin rays. These results fall in line with those described above for the unpaired fins.

Operculum.

On January 15, 1906, a set of six fish had a piece of the operculum removed, in order to determine whether the actual bony structures of the fish would regenerate. The piece removed was about one eighth of an inch broad and half an inch long, and was taken from the lower posterior border. Six weeks later two of these fish remained, but showed no signs of regeneration. A few days later, these also died. One fish with operculum cut as above was kept in a dish in the laboratory for four months, but at the end of this time showed no signs of regeneration.

The same experiment was tried with two sets of fifteen fish each at Wood's Hole. These fish were kept from June 20, 1906, to August 25, 1906, but at the end of this period showed no signs of regeneration.

Fish operated on in the above manner are sometimes difficult to keep alive since the gills are at first exposed to attacks of parasites and the nibbling of other fish. In a short time, however, the branchiostegal membrane spreads over and partly closes the opening.

Lower Jaw.

On March 23, 1906, pieces about one quarter of an inch long, were removed from the right-hand side of the lower jaws of thirty-six fish. Six weeks later, twenty-eight were still living. The jaws had grown again to such an extent that some of them could scarcely be distinguished from normal ones. Whether new bone took the place of that removed, was not accurately determined, though microscopic examination of sections of three of these new structures seemed to indicate that cartilage at least had regenerated.

Attempts were also made to remove larger portions of the jaw, or the entire jaw, but these always resulted in the death of the fish even if the wound healed. This may have been due to the difficulty the fish experienced in grasping and holding its food.

SCALES.

On June 20, 1906, the scales were removed from definite areas of a number of fish. This operation was performed in such a way as to injure as little as possible the underlying dermis, each scale being separately pulled out with a pair of fine forceps. Three sets of fifteen fish each were used in this experiment, each set of fish having the scales removed from a different part of the body. In three weeks time the new scales were coming in rapidly, some of them being one third the normal size, though very thin and soft. Two weeks later, the new scales were still only one-half to three quarters the length of a fully grown scale, though many were as broad. It appears then, that regenerating scales grow faster in breadth than in length. At this period or a little earlier the characteristic dotted pigment appears. At first the dots are comparatively few in number but continue to increase until the pigmentation, normal, for the position of the scale is attained. Thus each scale, from whatever region it is taken, is replaced by a new one pigmented exactly like the original. The fish in this experiment were kept ten weeks in all and at the end of that period the regenerated scales were practically normal in size, thickness and color though sometimes slightly irregular in shape.

LENS.

On June 21, 1906, the lens was removed from one eye of a number of fish. The operation was performed by slitting the cornea with a sharp scalpel and then gently pressing the eyeball with forceps until the lens popped out. In all thirty-six fish were used which were divided into two sets of eighteen each. In one of these sets the iris was slit in addition. These fish were kept until August 29, about ten weeks, when eleven fish remained in one set and nine in the other. When the lens is squeezed out some of the vitreous humor goes with it and after the operation the eyeball appears flattened. However the cornea heals over very quickly and the eye soon assumes its regular contour, differing only from normal apparently, in a slight dilation of the pupil.

The lost vitreous humor is apparently made good in some way. An examination of the eye at the end of the experiment (after ten weeks) revealed not the slightest indication of a new lens. Regeneration of the lens in *Triton* and *Salamandra*, has been obtained by Colucci ('91), Wolff ('95) and Fischel ('98). These results cannot then be extended to the teleost, *Fundulus heteroclitus*. However, it still remains to be shown whether the regenerative powers of the eye are not better developed in other species of teleosts.

Summary and Conclusions.

The general conclusions to be drawn from the foregoing experiments are the following:

1. In *Fundulus heteroclitus* the dorsal, caudal, anal, pectoral and pelvic fins will regenerate even when cut off close to the body, provided the proximal ends of the dermal fin-rays remain in situ. No regeneration takes place except when the stumps are present, and of sufficient size.
2. The operculum does not regenerate.
3. The lower jaw completes itself if a piece is removed.
4. The scales, if carefully removed, are quickly replaced by others.
5. The eye does not regenerate a new lens.

The results on the regeneration of fins support in general Broussonet's earlier work and that of subsequent writers. The ability to regenerate a portion of the jaw has been found by Spallanzani (1768) and others in the salamander. The lack of ability to regenerate a new lens in *Fundulus*, however is peculiar, since the salamander, an animal of a higher order, possesses it to a marked degree. It would be interesting in this connection to test the regenerative powers of the eyes of elasmobranches and ganoids, as being more generalized types than the teleosts.

In regard to Broussonet's statement that those fins which are the most useful, regenerate the quickest, it seems more probable that the conditions of the cut, as described for each experiment above, are the factors which determine the rate of regeneration, given equal conditions of temperature, food, vitality, etc. Indeed it is difficult to estimate the relative rate of growth, for in any one set of fish kept in the same tank and having the same fins removed, the differences in the rate of renewal are so great that some of the new fins may have completed half their growth when others are just beginning to show definite regeneration. Again, the caudal fin though obviously the most important in many fish does not regenerate, perceptibly, faster than the others. Finally, leaving aside the caudal it is difficult to say which fin is the most important. The observations which Osburn ('06) has recently made on the functions of fins seem to indicate that the pectorals are more useful than the dorsals and anals while my own results show that the former regenerate much slower. Broussonet was probably led to his statement concerning the relative rate of regeneration in different fins by his acceptance of the idea of regeneration as an adaptation. *A priori*, the caudal fin would regenerate, the most rapidly, on the hypothesis, since it is the most useful. The facts as I have tried to show, do not bear this out.

At this point the question naturally arises as to the relation between regeneration and liability to injury. It is true that the fins, extending out from the body as they do, are subjected to injury through brushing against obstructions, attacks of predatory animals, etc.; therefore, one might say, they acquired the power to regenerate as an adaptation to these conditions. But why, on the adaptation hypothesis, should the jaw regenerate so readily, when it is extremely improbable that this structure would ever be lost in a natural state? From the lack of regenerative power in the operculum and eye no conclusions can be drawn for or against this hypothesis, since these structures are not especially liable to injury under normal conditions. The power to regenerate scales, however, seems truly adaptive.

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October 6, 1906.

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NOTE ON THE GEOTROPISM OF ARBACIA LARVÆ.

E. P. LYON.

As soon as the blastulæ of the sea urchin begin to swim, they come to the top of the water. They remain in this locality during the blastula and gastrula stages but become scattered when they reach the pluteus condition.

The gathering at the top is not a light effect, for it takes place in total darkness.

The gathering is not due to lack of oxygen, for the larvæ go up in an inverted test-tube filled with sea water and remain at the top a long time. With the dense gathering of thousands of blastulæ at the top of such a tube the gradient of increasing oxygen supply must be from above downward, yet the larvæ do not go down. A narrow tube closed at one end was nearly filled with water containing young arbacia. An air bubble was introduced into the tube about the middle of its length. The tube was then placed with the closed end directed up. The larvæ went up in both ends of the tube, those above the bubble going directly away from the oxygen supply.

Experiments in tubes kept at constant temperature showed that the gathering was not due to currents in the water. Nor was the gathering due to the larvæ being lighter than sea water. Their specific gravity, on the contrary, is considerably higher, being about 1.060.

That the gathering of larvæ at the top is a true gravity effect is indicated by their moving against a centrifugal force. The best way to demonstrate this is by placing sea water containing many larvæ in rather long (say, 25 cm.), narrow tubes. On rotating these carefully, it will be found that beyond a certain radius (depending on the rate of rotation) the larvæ are precipitated, the acceleration in these parts of the tube being greater than they can swim against. Inside this critical circle the larvæ will be found to have moved toward the axis of rotation and to be gathered in dense crowds.

Paramœcia will rise to the top of a solution of the same density as themselves and in which they have, therefore, no weight. This fact, to my mind, is proof that gravity acts within the organism and not through the relative densities of water and organism. With the larvæ of arbacia, however, I was unable to get any definite gatherings in a solution of gum arabic and sea water of the same density as the larvæ. The viscosity of this medium is so great that such minute ciliated organisms as those under consideration can hardly move through it.

While convinced, therefore, that the gathering of the arbacia larvæ at the top of the water is a gravity effect, I am unable at the present time to say how gravity brings about the response. The possibility that the orientation is purely passive, a buoyancy effect, must be kept in mind, as well as the various theories proposed by Jensen and Davenport. However, on the basis of my paramœcium work, I consider it most probable that gravity acts directly on the cells.

The blastulæ from centrifugalized eggs have all the pigment on one side and are therefore presumably heavier on that side. Later examination shows that this pigment may be in any part of the gastrulæ or plutei. Such larvæ, although, as stated, presumably unbalanced, nevertheless come to the top of the water. I consider this observation opposed to the theory of orientation by buoyancy, for if the heavier portion were always directed down and this portion sustained no relation to the direction of locomotion, the organisms ought to be scattered through the water.

NOTE ON THE HELIOTROPISM OF PALÆMONETES LARVÆ.

F. P. LYON.

The striking peculiarity of the light reaction of these larvæ is that they orient themselves with the anterior end away from the light and then swim backward toward the light. If orientation alone is the criterion of a tropism, these organisms are negatively heliotropic. But they gather on the side of the disk toward the light and as ordinarily observed would surely be considered positive.

Blue light produces these effects as strongly as daylight. Red light is almost ineffective.

The orientation of the larvæ is usually extremely precise. The instant that light is admitted to a dish containing the larvæ, they all turn so that their heads are directed away from the source of light and the body axis is in the line of the rays. In many the abdomen is strongly extended so that the body axis is a straight line. These swim backward practically in a straight line. Very frequently they swim on their backs in a perfectly straight course to the light side of the dish. Others swim with ventral side directed down.

Besides the individuals which hold their bodies extended and exhibit very accurate orientation there are many which swim with the abdomen more or less flexed ventrally. These are inclined to rotate, but their general course is quite exact toward the light.

A study of the water currents, when India ink was added, showed that the backward movement was the result of strong forward beats of the swimmerets. The details have not been studied. The animals can move forward, but this form of locomotion is quite different from the backward type, being accomplished by a series of very rapid jumps or plunges. So quick were these forward movements that I could not accurately observe how they were accomplished. I believe that they were due to strong extension of the tail, the animal first bending the body ventrally and then suddenly extending it. The jumping reaction occurred on strong stimulation of any kind : for example, on throwing light suddenly into the dish. The orientation with the head away from the light was accomplished by one or more leaps, but so accurately that one could hardly speak of trial and error. When the animals in swimming backward toward the light suddenly hit any obstruction, the springing reaction carried them momentarily away from the light. But they immediately began, under the influence of the swimmerets, to move backward toward the light. So it would often happen that on reaching the side of the vessel the larvæ would strike, jump away, swim back, jump away again, and so on many times without losing their orientation in the direction of the light rays.

It was therefore of some interest to know how these larvæ would behave if the sense of response to light could be reversed. I have made only a beginning of this study.

The effects of raising the temperature were not clear. For the most part the leaping reaction predominated. The animals darted rapidly about and lost their orientation to light.

Better results were obtained by diluting the sea water. If the larvæ were placed in a mixture of 50 c.c. distilled water and 100 c.c. sea water, a considerable proportion of them went to the negative side of the dish. This they did without changing their orientation. In other words they now employed the forward instead of the backward type of locomotion and thus arrived at the side of the dish away from the light. Often there seemed to be a conflict between the two methods of locomotion, the animals oscillating back and forth like shuttles, but always with the heads away from the light. Equal quantities of distilled and sea water gave even more pronounced results.

The responses were not always so regular and machine-like as I have described. Some of the more pronounced "negative" individuals moved by an irregular, mostly sidewise motion away from the light. Taken as a whole the "negative" effect secured by diluting the sea water was less precise and definite than the "positive" reaction. Also most of the "negative" individuals were only temporarily so.

On returning negative individuals to the undiluted sea water they at once swam to the light side of the dish, but without changing their orientation, *i. e.*, with head directed away from the light.

The question of nomenclature which these observations suggest may well await further investigations. The experiments here recorded were made at the Marine Biological Laboratory at Woods Hole.

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CHROMOSOME RELATIONS IN THE SPERMATOCYTES OF ONISCUS.

M. LOUISE NICHOLS.

Recent work on the germ cells of animals has shown that the chromosomes contained within one nucleus frequently are not all of the same size. It has been known for some time that in insects and spiders one or more of the chromosomes, the so-called accessory, is smaller than the others and also differs in its behavior. With regard to chromosomes other than the accessory, Sutton,[1] in his studies on the spermatogenesis of *Brachystola* discovered a well-marked difference in size. Montgomery's[2] studies of the chromosomes of various Hemipteran species also revealed a variation in size of chromosomes occupying the same nucleus, and McClung,[3] in a recent paper on the spermatocytes of Orthoptera has described a remarkable structure, strikingly larger than the other chromosomes, which he has named "multiple chromosome," and which he interprets as being formed by the association of a number of chromosomes. The still more recent work of Morgan[4] and Stevens[5] on aphids indicates much the same thing. Morgan studied the eggs of Phylloxerans and shows in his figures of *Phylloxera caryæ-globuli* a distinct difference in the size of the chromosomes. Miss Stevens investigated a number of species of plant lice and concludes that each species is characterized by a definite number of chromosomes.

Chromosomes of the same nucleus may differ not only in size but also in shape. In a paper published in 1902[6] I showed that in the equatorial plate of the first maturation division of the male reproductive cells of the wood-louse, *Oniscus*, there occur chromosomes of three kinds. Some are straight or dumb-bell shaped, others are crescent shaped. In these two forms the component parts of the bivalent chromosomes lie end to end. A third form exists in which the univalent chromosomes are joined not end to end, but side by side. In each type a split may be observed running the length of the chromosomes and the first division is therefore reducing. In the prophase the third type is represented usually by rings, occasionally by V-shaped structures (Figs. 7 and 14), while the crescents appear as curved rods and the remainder as straight or dumb-bell shaped rods. The reduced number of chromosomes, as far as could be determined, is sixteen. Of this number two have the ring form, two are crescents and the others are straight or dumb-bell shaped. The chromosomes vary somewhat in size, but in *Oniscus* the differences are not so great as in some of the insects before mentioned. The largest have the ring form, the smallest are straight (Figs. 1, 2, 4, 17, 18). Sometimes one of the two rings is complete or nearly so, the other incomplete (Figs. 1, 11). The rings lie on opposite sides of the nucleus, sometimes directly opposite, in other cases apparently shifted to one side or the other (Figs. 1, 2, 3, 8, 9, 11, 15, 16). Since they are the largest and most easily recognized of the nuclear elements they form convenient points of localization.

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[1] W. S. Sutton, "On the Morphology of the Chromosome Group in *Brachystola magna*," BIOL. BULL., 4, No. 1, 1902.

[2] T. H. Montgomery, Jr., "A Study of the Germ Cells in Metazoa," *Trans. Am. Phil. Soc.*, 20, 1901.

[3] C. E. McClung, "The Chromosome Complex of Orthopteran Spermatocytes," BIOL. BULL., 9, No. 5, 1905.

[4] T. H. Morgan, "Male and Female Eggs of Phylloxerans of the Hickories," BIOL. BULL., 10, No. 5, 1906.

[5] N. M. Stevens, "Germ Cells of Aphids," Carnegie Inst., May, 1906.

[6] M. L. Nichols, "Spermatogenesis of *Oniscus asellus*, Linn.," *Proc. Am. Phil. Soc.*, 41, No. 168, 1902.

The crescent form is the next most easily recognizable. Of this type there are likewise two, one of them sometimes more strongly curved than the other. They also occupy opposite sides of the nucleus and at points between the positions occupied by the rings (Figs. 3, 4, 8, 9, 10, 11, 15, 16, 19).

The relative position of the rings and crescents is more readily determined than that of the rods, because they are not so numerous and are also distinguished by their shape. Repeatedly however I have observed an arrangement of three or four straight chromosomes in a row (Figs. 9, 12, 17). One of the smaller of the straight chromosomes, too, is often seen lying not far from one of the crescents (Figs. 8, 13, 17, 18).

These facts point to an individuality of the chromosomes and also indicate a tendency to localization in the nucleus. The evidence for this is further strengthened by the manner in which the chromosomes develop from the resting nucleus. The resting nucleus is a network formed by the gradual lengthening and anastomosis of threads which retain their individuality until just before the formation of the nuclear membrane. From it arise the chromosomes of the first maturation division by a breaking apart of the threads of this network and their gradual shortening and thickening.

The question presents itself whether these relations of form and position of the chromosomes are peculiar to *Oniscus* or characteristic of the land isopods as a group. Two other genera I have examined, *Porcellio* and *Armadillo*. The spermatogenesis of these is similar to that of *Oniscus*, but the cells are smaller, in *Armadillo* so small as to make an examination of the chromosomes very difficult and uncertain.

The cells of *Porcellio* more nearly approach those of *Oniscus* in size and I was able to compare the prophase of the first division in the two animals. Although the chromosomes of *Porcellio* are smaller than those of *Oniscus*, the peculiarities of shape and arrangement are similar. In *Porcellio* also are present two rings lying opposite each other with a crescent between. More than one crescent in a nucleus, however, I have not seen. One at least of the straight chromosomes appears considerably smaller than the others and, as in *Oniscus*, frequently lies not far from the crescent (Figs. 5 and 6). I was not able to determine the number of chromosomes in *Porcellio*, as my preparations contained no spermatocytic mitoses. Besides the smaller size of the chromosomes would make an accurate determination even more difficult than in *Oniscus*. As far as can be judged from the appearance of the prophase, the number would seem not to be very different in the two genera.

The presence of chromosomes of the three forms described within the same germ nucleus is not then peculiar to *Oniscus*, but probably is characteristic of the group, the genera being visibly distinguished from each other by differences in size of the chromosomes and possibly by slight differences in the shape of homologous chromosomes.

Comparing the results obtained for various animals and plants, it seems safe to conclude that any correlation existing between the shape of the chromosomes and the tangible bodily characteristics is not very close nor exact. Species quite distinct from each other may possess chromosomes of almost the same shape and size. Especially is this true of plant species. On the other hand, species closely resembling each other may have chromosomes of perceptibly different shape or size. This is even more obviously the case with the number of chromosomes. In some instances a difference in number may be accompanied by difference in sex,[1] in others by specific differences.[2] Again the same number may characterize groups as widely different as angiosperms, mammals and insects [3]; while related species of the same genus may differ in number.

Nevertheless in a general way there appears to be some correspondence between the bodily attributes of a group and the character and behavior of the chromosomes of that group. Chromosomes of a particular shape are more characteristic of certain groups than of others. Thus, the form in which the limbs of the bivalent chromosomes are twined about each other is frequently found in flowering plants and also in amphibia, the ring form is particularly apt to occur in arthropoda and a shape somewhat like a shepherd's crook, with a slender body and thickened, curved extremity, is often seen in mollusks and worms. Again the peculiar chromosome known as the accessory so far has not been found outside of the Tracheata.

No doubt the shape, possibly also the number of chromosomes, is determined by complex physical and chemical conditions which may not have any direct relation to visible bodily characters and two chromosomes of the same shape and size may contain potentialities of quite different nature, just as an apple and its waxen imitation, although of the same size and shape, have very diverse properties.

A parallel condition may be seen in the relation of the shape and size of the egg to the bodily characters of the organism developing from it. Forms differing as widely as fish and echinoderms may have eggs of about the same size and shape, but of varying potentialities. Yet here, too, there are peculiarities of constitution and behavior characteristic of certain groups rather than of others. There may be as good ground for determining the relationship of organisms by the behavior of chromosomes as by the character of cleavage in the egg, but in the former case the handicap is severe because of the minute size of the elements and the fact that changes of important nature may take place in the constitution of the chromosome without producing any effect tangible for modern microscopic methods. If, however, specific or other differences happen to coincide with visible variations in the chromosomes, such objects form convenient points of departure for the study of such problems as the determination of sex and the relation of paternal and maternal chromosomes in the cells of hybrid offspring.

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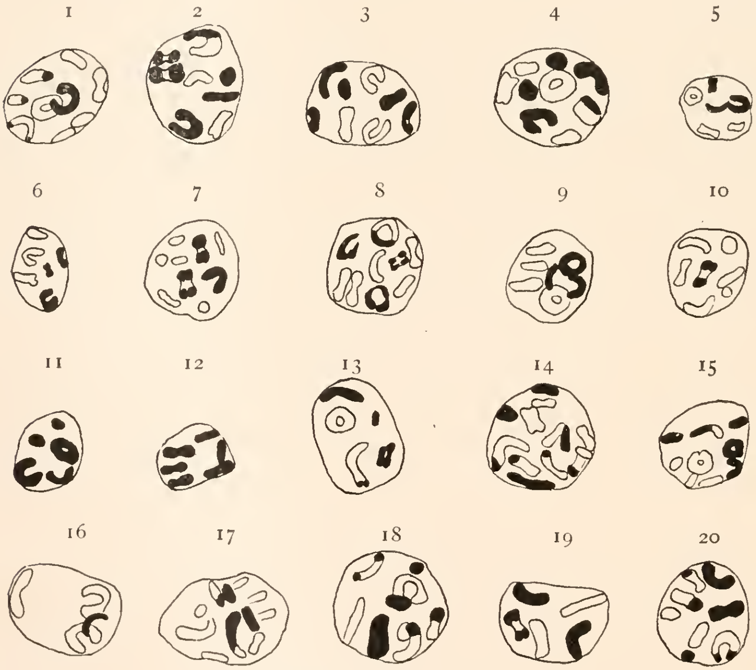
[1] E. B. Wilson, "The Sexual Differences of the Chromosome Groups in Hemiptera," *Jour. Exp. Zool.*, III., No. 1, 1906.

[2] Montgomery, Morgan, Stevens, *loc. cit.*

[3] E. B. Wilson, "The Cell in Development and Inheritance," p. 206, 1900.



Explanation of Figures.

With the exception of 5 and 6, the figures are prophases of the first maturation division of *Oniscus asellus* Linn. Nos. 5 and 6 are from *Porcellio*. The drawings were made with the camera lucida, Zeiss oc. 6, obj. $\frac{1}{12}$ imm. at the level of the table.

A PRELIMINARY NOTE ON ANT BEHAVIOR.

C. H. TURNER.

There is a wide-spread belief among living entomologists that ants slavishly follow the odor trail and Bethe insists that ants are mere reflex machines. For something over three years I have been conducting a series of experiments on the behavior of ants, the results of which do not harmonize with the above view. These experiments, which were performed upon a large number of southern species and a few northern species, show conclusively:

1. That the movements of worker ants are not controlled by tropisms.

2. That ants are not guided by a homing instinct.

3. That ants are enabled to take long trips by learning, by experience, the way and retaining what they have thus acquired. They learn the way in the same manner that vertebrates learn to open problem boxes and to run mazes.

4. That ants do not as slavishly follow the odor trail as is supposed.

5. That Sir John Lubbock was right when he said: "In determining their course ants are greatly influenced by the direction of the light."

6. The color of the pathway has little or no effect upon the home-going of ants.

7. That Dr. Wheeler is right in stressing the high development of the female; for the winged females often take part in the regular duties of the nest. I have had them learn the way home from new situations and assist the workers in carrying the pupæ home.

8. That the males are stupid and seem unable to solve even the simplest of problems. They seem to be more or less heliotropic.

9. The major workers of *Pheidole*, which Ernest Andre claims function as soldiers and do not take any active part in the ordinary work of the nest, frequently assist the workers in making

excavations; and, occasionally, assist in conveying pupæ from one place to another. I have never noticed one continue to carry pupæ for any considerable length of time.

10. Ants are affected by olfactory, optic, tactile, kinesthetic and probably auditory stimuli.

11. Ants seem to have fairly definite impressions of direction, both in horizontal and vertical planes, and of distance.

12. Ants have associative memory.

13. Such cases of division of labor as Romanes — quoting from Moggridge, Lespes, Belt and Herr Gredler — describes in his "Animal Intelligence," are to be looked upon as cases of incidental coincidence rather than as examples of mutual coöperation.

14. Ants can be trained to do certain simple things in the same way that vertebrates are trained.

15. In their activities ants display marked individual variations

In this preliminary paper, space forbids a discussion of the data upon which the above statements are based; that will be done in a paper which I expect to publish in the spring or summer of 1907. In this communication I merely desire to give some of the evidence that warrants the fourth and fifth statements.

In these experiments I used a card-board stage six inches square, from which a card-board incline, three fourths of an inch wide, led down to the island upon which the nest of the ants was confined. A great many pupæ and worker ants were placed on this stage. After many random movements the ants learned the way from the stage to the nest and back. The way once learned, the ants continued to work until all of the pupæ had been carried into the nest. I then replaced the ants and pupæ on the stage. The pupæ were again carried home. This was repeated over and over again; until, by their actions, I concluded that the ants were thoroughly acquainted with the way down the incline to the nest. Then a second incline was so placed as to lead from the opposite side of the stage to the Lubbock island. If, after a lapse of a few minutes, no workers descended this second incline, I concluded that the workers thoroughly knew the way down the other path. I then placed the first incline, which had become scented by the foot-prints of the ants, in the place where the new incline had been and placed a new unscented incline in the place formerly occupied by the one the ants had been traversing. Thus there was an unscented path in the position of the old trail and the old familiar scented path was in a new position.

Now if ants are guided home solely by the sense of smell, they should either have spent approximately as much time learning the way down the new incline as they did before; or else, in their random movements, they should have happened upon the scented incline and gone down it. In reality, they did neither of these things. They went almost immediately down the unscented incline which occupied the position in space formerly filled by the incline down which they had been carrying pupæ to the nest.

Similar experiments, yielding the same results, were conducted with marked individual ants.

It will be seen at once that these experiments disprove not only Bethe's double polarized trail hypothesis but also Wasmann's assumption that their odor tracks have for ants an odor-shape which guides them home.

According to the current belief, ants going to and from my stages should have followed the same path. But experiments, conducted with marked individual ants, showed that this was not always the case. I found:

1. That the ant had to learn the way, not only from the stage to the island, but also from the nest back again to the stage and that it usually required more time to solve the second problem than it did to solve the first.

2. That sometimes an ant would regularly descend to the island along the top side of the incline and ascend to the stage along the under side of the same incline.

3. I have noticed a worker regularly descend from the stage by way of the incline and ascend the stage by way of its central support.

4. I have had ants ascend to the stage by way of one incline and descend by the other.

5. In passing from the nest to the foot of the incline the ant did not always follow the same path.

6. I have known an ant to regularly drop from the stage with a pupa and carry it to the nest and then return to the island, mount a section lifter, which I presented, and rest quietly thereon until it had been conveyed to the stage. Then it would step off, pick up a pupa and drop from the stage. This was repeated over and over again by the ant.

More than a quarter of a century ago Lubbock claimed that ants were influenced by the direction of the rays of light. These experiments have been either overlooked or ignored by recent continental writers. Although Sir John Lubbock's experiments were not so planned as to exclude the possibility of the effect noticed having been produced by heat or change in the intensity of light, yet it is hard to see any reason why they should have been so completely ignored by European writers. My experiments on the effect of light upon the home-going of ants, which were conducted under perfectly planned conditions of control, resemble very much certain of Lubbock's experiments.

Two kinds of tests were conducted: experiments with ants working in concert, and experiments with marked individual ants working alone.

In experiments of the first type a card-board stage was used, from which a card-board incline led down to the island. A 16 c. p. incandescent lamp was placed near the side to which the inclined plane was attached. After the ants had thoroughly learned the way home, a new incline was attached to the stage on the side opposite the one to which the old incline was still attached. If, after a lapse of five minutes, no ants went down the new incline, conditions were considered right for the test. The light was then transferred to the opposite side of the stage. In each test the halting movements of the ants showed that they were much disturbed. In most cases, some of the ants would finally go down the new incline; and in a few cases, after the lapse of several minutes, all of the ants went down the new incline. The above described experiments with ants working in concert were conducted with several different species.

The individual experiments to test the same thing were conducted with *Formica fusca* var. *subsericea* Say and *Myrmica punctiventris* Rog. The apparatus was arranged in the same manner as for ants working in concert; but, marked ants were used and only one was allowed to work at a time. In all essential points, the results harmonized with those obtained from ants acting in concert. In most cases, however, after a greater or lesser lapse of time, the ant would usually find its way down the old incline to the nest; and, after a greater lapse of time, find its way back again to the stage.

That the above effect was not due to heat was proven in the following manner. At the right and left of the stage used was placed a tall museum jaw .34 cm. × 16 cm. × 17 cm., filled with distilled water. These heat filters were so adjusted as to have their broad sides parallel to the edges of the stage. At the beginning of the experiment, a 32 c. p. incandescent lamp was placed behind one of these filters (usually behind the one that was near the incline). After the ants had traveled the path long enough to make the trips regularly and rapidly, the lamp was transferred to behind the opposite filter. In every case the workers were much disturbed in the manner stated above. This was repeated with seven different colonies of *Formica fusca*. Since the heat had been excluded, it is evident that the disturbance was the result of some form of light stimulus.

To determine whether the intensity or the direction of light was the determining factor, the experiment was slightly modified. The stage and incline were arranged as before. Sometimes the heat filters were used, but more often they were not. To furnish illumination, four different candle powers (4 c. p., 8 c. p., 16 c. p., 32 c. p.) of incandescent lamps were used, one at a time, in a darkened room. At the beginning of the experiment a lamp of a certain candle-power was placed near the side of the stage to which the incline was attached. After the ants had thoroughly learned the way home a different candle-power was substituted for the first. After the lapse of a few more minutes this lamp was transferred to near the opposite side of the stage. Shortly it was returned to its former position. A few minutes later a different candle-power was substituted for this. This performance was repeated over and over again until each candle-power had been used one or more times.

It was found that substituting a lamp of one candle-power for a lamp of a different candle-power had no disturbing effect upon the actions of the ants; but that by any marked change in the angular position of the light, no matter what the candle-power, the ants were much disturbed. This forces us to the conclusion that the direction of the rays of light, when present, plays a prominent rôle in the home-going of ants.

Zoölogical Laboratory,
University of Chicago,
November 10, 1906.

ON THE DEVELOPMENT OF PARASITIC COPEPODS.[1]

PART I.

J. F. McCLENDON.

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The work herein presented was begun at Wood's Hole, 1904, with a view of determining the effects of pressure on the eggs of parasitic copepods. Owing to technical difficulties, three summers have passed with few experimental results, and in consequence the present paper is chiefly of a morphological nature.

I obtained parasitic copepods from fish caught in traps of the Marine Biological Laboratory and the U. S. Fish Commission, at Woods Hole. It was usually necessary to go to the trap to get them fresh, or in the case of some Caligidæ to catch them before they left the fish.

Most of the work was done on *Pandarus sinuatus* Verrill, *Læmargus muricatus* Kröyer (collected by Dr. Conklin), and a new species (text Fig. 3) of the family Dichelesteidæ and of a new genus near *Kröyeria* (according to Prof. Charles B. Wilson) which I will call throughout this paper the dichelestid. The eggs of *Pandarus sinuatus* are the most difficult to handle, being very flat and thin and pigmented, but I used them because this is the most common species at Woods Hole. I used the eggs of the following for comparison (arranged according to the classification of Claus):

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[1] Thesis accepted by the Faculty of the Department of Philosophy of the University of Pennsylvania toward the degree of Doctor of Philosophy.

- Caligidæ:
  - *Caligus bonito* Wilson (and several undetermined species of *Caligus*).
  - *Caligus rapax* Milne Edwards.
  - *Lepioptherius edwardsi* Wilson.
  - *Perissopus communis* Rathbun.
  - *Nesippus alatus* Wilson.
  - *Cecrops latreillii* Leach.
  - *Læmargus*[1] *muricatus* Kröyer (and an undetermined species of *Læmargus*).
  - *Philorthagoriscus serratus* Kröyer.
  - *Pandarus sinuatus* Verrill.
- Dichelesthiidæ:
  - *Anthosoma crassum* Abilguard.
  - *Kröyeria ?*
  - *Eudactylina nigra* Wilson and *E.* sp.?
- Lernæidæ:
  - *Penella.*
- Chondracanthidæ:
  - *Chondracanthus.*
  - *Sphyrion ?*

For the determination of species I am indebted to Prof. Charles Branch Wilson, of Massachusetts State Normal School, Westfield, Mass., who has been very kind in his interest in my work.

After my work was nearly finished I received the material collected at Woods Hole in 1899 by Prof. Edw. Rynearson, of Pittsburgh, with a view of working on this same subject, and which he kindly turned over to me. I wish to express my thanks for this abundance of material, which allowed me to confirm many points on which my material was scanty and to add some new ones.

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[1] The word *Læmargus* was applied to a copepod and a shark in the year 1837 and as yet it is disputed which should claim priority.

I am indebted to Dr. Conklin for constant guidance and assistance besides the general direction of my work, and to Mr. Kribs for the use of Zeiss apochromatic lenses.

Dr. Formad and Dr. Fischelis rendered me invaluable service in translating Russian.

I am under obligations to the Carnegie Institute for a table in the Marine Biological Laboratory, Wood's Hole, 1904, and to the U. S. Fish Commission for a table in the U. S. F. C. Laboratory, Wood's Hole, Mass., 1905–6.

The material was fixed in Flemming's fluid, picro-acetic, or corrosive-sublimate-acetic. The first gave the best fixation but the second was the most convenient. Heidenhain's iron hæmatoxylin followed by various counter-stains, and Hermann's safranin-gentian violet were used most frequently.

II. Historical.

The ground covered in this paper was almost entirely untouched by earlier workers as regards the particular genera treated, and I will consider here only some papers on related forms.

The free living copepods have long been favorite objects for study. Gruber ('79) described their reproductive organs. Weismann and Ischikawa ('88 *a*, *b*) and Ischikawa ('92) used them in studying the question of reduction in number of chromosomes. Haecker ('91, '92 *a*, *b*, '94 *a*, *b*, '95 *a*, *b*, '97, '02) found them favorable objects for study of oögenesis, maturation, reduction, and unsymmetrical mitoses. Later, Rückert ('94, '95 *a*, *b*, *c*) studied oögenesis, maturation, and reduction in these.

Of the parasitic and half parasitic copepods, Heider ('79) notes the spermatogenesis of *Lernanthropus*, and Giesbrecht ('82) the oögenesis of the Notodelphidæ.

Charles Branch Wilson in his monograph on the Caligidæ ('05) describes the anatomy of the reproductive organs and the life history.

III. Anatomy of the Reproductive Organs.

In *Pandarus*, *Caligus* and allied forms the two ovaries lie in the head (Fig. 1) and the two oviducts run backwards to the genital segment, where they are much convoluted and increase greatly in diameter and each communicates by an opening, the os uteri, with an egg string that trails behind the animal. The ovary is formed of a much convoluted cord of cells in a single linear series, small at the oögonial end and gradually increasing in diameter until it passes into the oviduct. As these cells (oöcytes) grow in the ovary they become much compressed in the direction of the long axis of the cord and the cell boundaries almost completely disappear. Passing into the oviduct, the oöcytes continue to grow and soon their boundaries reappear, and they become more flattened as they grow larger. There are at least two broods in the oviduct at once, caused by the periodic activity of the ovary. The younger brood extends some distance into the genital segment and is sharply marked off from the older brood, which occupies the last coils of the oviduct and consists of oöcytes that have nearly or quite completed their growth. As the oöcytes pass out through the os uteri they are fertilized from the seminal receptacles (Fig. 2, *sr*) and surrounded with secretion from the cement gland (*c.g.*) which forms the wall of the egg string. The eggs are distorted when passing through the thorax, but in most cases regain their symmetrical form. Embryos in the egg strings have their heads turned latero-ventrally and their ventral surfaces anteriorly, in relation to the mother. Occasionally one finds an embryo reversed, or partially rotated.

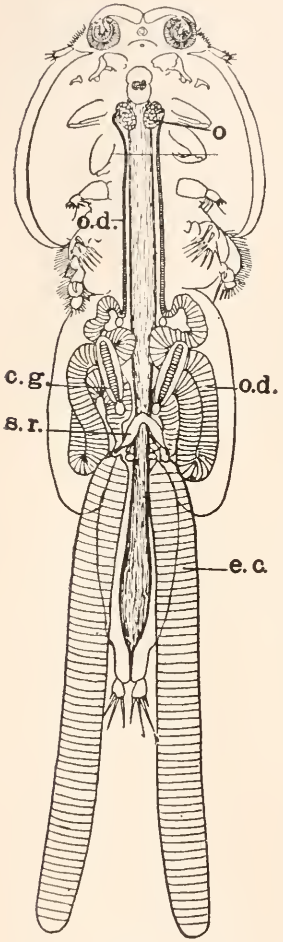


FIG. 1. Female reproductive organs of Caligus bonito. (Drawn by Emerton from Wilson's *Caligidæ.*) *c. g.*, cement gland; *e. c.*, external egg cases; *o*, ovary; *o. d.*, oviduct; *s. r.*, semen receptacle.

In the male the same general arrangement of reproductive organs exists, save that the seminal receptacles are absent and the distal ends of the vasa deferentia are enlarged and become the spermatophore receptacles. The cement glands secrete the material for the walls of the spermatophores.

In the dichelestid (FIG. 3) the genital segment is very much elongated and the oviducts are not convoluted. The ovaries have been carried backward until they lie in the anterior end of the genital segment and the oviducts pass forward to the anterior end of the thorax, then backward to the attachment of the egg strings. In other respects the description given for *Caligus* will hold for this species. The older brood of oöcytes occupies the posterior two-thirds of the oviduct. When one brood passes into the egg string the distal eggs of the remaining brood have nothing to press against and tend to round up and become distorted. The thickness of the oviduct at its posterior end varies somewhat, depending on the number of eggs produced in one brood, and the size of the eggs. When fewer, the eggs are thicker, and when more numerous, thinner. The egg string is more slender than the posterior portion of the oviduct, and the contained eggs are, therefore, thicker.

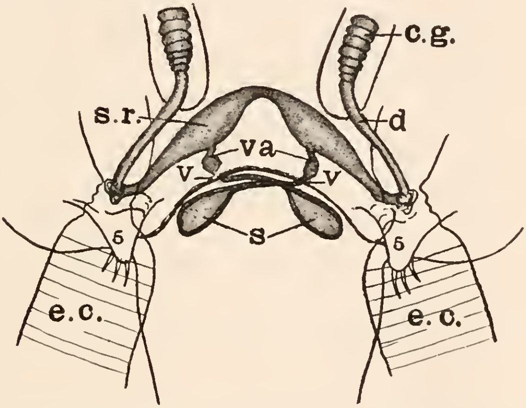
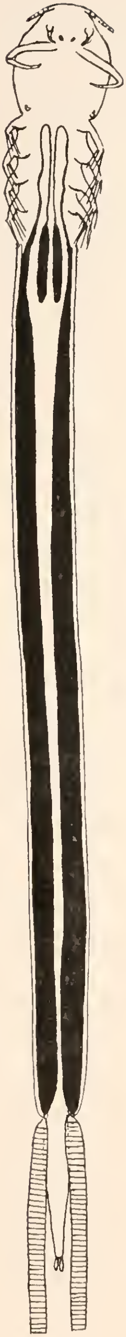


FIG. 2. Semen receptacles and vagina of a female *Lepeophtheirus*. (Partly after Claus.) *c. g.*, cement glands; *d.*, cement gland duct; *e. c.*, egg cases; *s.*, spermatophores; *s. r.*, spermaries; *v.*, vulva; *va.*, vagina; 5, fifth legs. (From Wilson's *Caligidæ.*)



In *Læmargus* the egg strings are packed in loops undér broad lamellar coverings.

IV. Oögenesis and Maturation.

1. *Oögenesis.*

A. The Dichelestid. — The oögonia (Fig. 1) are very small isodiametrical cells. The nucleus is spherical and contains chromatin granules in a peripheral linin reticulum. Minute nucleoli are also embedded in this reticulum. The cytoplasm stains deeply. I have found but few oögonial mitoses in this species although I have sectioned over a hundred females; the number of chromosomes is the same as in the primary germ cells of the embryo (16) and *twice* the reduced number (8).

The primary oöcyte is readily distinguished from the oögonium by its nucleus being about half the size of the latter (when first formed). The earliest stage I have is the late telophase, in which the chromosomes are drawn together as though at the pole of the spindle, yet the whole mass is surrounded by a nuclear membrane (Fig. 2). The nuclear membrane is therefore formed of the fused linin sheaths of the chromosomes (or from cytoplasm ?). This mass is placed excentrically in the nucleus and the individual chromosomes are so pressed together or fused that only their ends sticking out can be distinguished separately, so that it resembles a "synapsis." The cytoplasm stains deeply. The oöcytes are arranged in single linear series as stated above and are pressed and flattened one against the other; the nuclei are close to the free surfaces of the cells, and that edge of an oöcyte containing the nucleus is thicker than the opposite edge. Soon the chromosomes swell and the chromatin becomes

Fig. 3. The *Dichelestid*, ventral view of ♀. The ovaries and oviducts are solid black. Portions of the egg strings are still attached.

dispersed as granules in a peripheral achromatic reticulum, as in the oögonia (Fig. 3). Minute nucleoli appear in this reticulum. The boundaries between the oöcytes become so faint as to be no longer everywhere perceptible. At the same time the cytoplasm begins to increase in volume and the diameter of the egg cord to increase. This process continues until the egg cord passes into the oviduct, and for some time thereafter. When the oöcytes have traversed about a fifth of the oviduct, the cell boundaries reappear and the nucleus migrates to very near the center, there forming an enlargement in the oöcyte (Fig. 4). The reticulum and the three to four nucleoli have grown considerably with the growth of the nucleus. A few yolk spherules and fewer oil globules appear and grow to considerable size. For some time growth of the oöcyte consists almost solely in the addition of yolk spherules and oil globules and this continues until the yolk almost obliterates the cytoplasm and closely surrounds the nucleus. I have seen these spherules and oil globules separated from the protoplasm with a high speed centrifuge (kindness of Dr. Lyon), the former are heavier and the latter lighter than the protoplasm.

B. Læmargus muricatus.—The oögenesis resembles that of the dichelestid. In the oögonial divisions there appear to be sixteen chromosomes, but these are set so close together that I cannot be sure by actual count.

In the growth period the reserve materials, yolk and oil globules, are laid down in ways that show them to be of quite different consistency. The oil globules (dissolved out in sections) appear as minute points that grow in size but always retain a spherical shape. The yolk substances, at least some of them, are laid down as thin discs. A disc receives new substance on its flat surfaces only, and the layers of substances are alternately chromatic and achromatic in staining qualities (when much destained after certain stains). Some of these piles of discs become spherical, others oblong by addition of more discs. The constituent layers do not mix but remain separate until dissoved in the segmenting egg or developing embryo. The oil globules are relatively few in number and large in size. The yolk bodies vary greatly in size, none of them reaching the size of the oil globules.

C. Pandarus sinuatus. — The oöcyte of *Pandarus sinuatus* differs from that of the dichelestid in that a single nucleolus is formed, about equal in bulk to that of the several nucleoli of the latter species together. The oöcyte of the former is much more compressed (thinner) than that of the latter.

2. *Maturation.*

A. Pandarus sinuatus. — At the end of the growth period the nucleus becomes irregular in outline which gives it a shrunken appearance. The nucleolus becomes vacuolated and the nuclear sap intensely staining. The location of chromatin cannot be made out very well, but in thin sections chromatic threads can be seen radiating from the nucleolus. Soon the nuclear sap fades enough to show that the chromatic threads have split (Fig. 6). The nuclear wall is dissolved. Sometimes the vacuoles in the nucleolus increase very much in size and fuse into one. In preparations of the entire oöcyte the chromatic thread can be seen to be divided into eight double chromosomes (Fig. 5). By shortening of these double-rod-shaped chromosomes, ring-shaped chromosomes are formed (Fig. 7). A transverse constriction transforms the diad into a tetrad, that is a ring constricted at four equidistant points, the two opposite constrictions, representing the divisions between the original rods, being deeper than the other two. The spindle when first formed is longer than the shortest diameter of the egg. It is similar to that found in free living copepods, having no polar rays. The dense protoplasm at each end of the spindle (Figs. 7 and 9) may possibly be derived from archoplasm of preceding divisions, and it shows a striking resemblance to the pole plates of the dividing nucleus in Protozoa. The spindle is at first parallel to the flat surfaces of the egg and rotates to an almost vertical position (Fig. 8). The first polar body is very small and is extruded between the egg and its neighbor at about the center of the flat surface that is posterior (in relation to the mother) (Figs. 9 and 10). The second polar spindle is smaller than the first. It is at first parallel to the flat surfaces of the egg and rotates nearly to a perpendicular position under the first polar body (Figs. 9 and 10). I have not seen the second polar body being cut off and have distinguished only one polar body in later stages, which on account of its size I regard as the first polar body or both polar bodies fused (Fig. 49, *P*). It may be that the second polar body remains in the egg as in *Cyclops* and is not easily distinguishable, or more probably it is extruded immediately under the first polar body and the two being pressed together and becoming very flat, appear as one. The chromosomes in the first polar body do not swell and fuse to form a nucleus.

I have made no observations on the entrance of the sperm. Vide under Polarity of the Egg.

B. Læmargus muricatus (Fig. 18). — Maturation in this species is very similar to the process in *Pandarus*. The disintegration of the single large nucleolus shows many stages which appear to be peculiar to this species. A number of vacuoles (globules of achromatic fluid) appear in it, and these sometimes fuse into a single mass. The chromatic substance does not remain as a continuous peripheral layer but rounds up into four or more masses whose inner surfaces are hemispherical and outer surfaces form portions of the general surface of the nucleolus. In some cases there are two fluids (one colorless and the other staining very faintly) formed within the nucleolus. The colorless fluid forms a large sphere in the center and the faintly staining fluid occupies the periphery and contains spheres of the original chromatic substance of the nucleolus. The chromosomes do not go as far in metamorphosis as in *Pandarus*. In the equatorial plate the chromosomes appear as short double rods (Fig. 18), and if we regard the spireme formed of chromosomes joined end to end and cut into half the usual number of pieces the first maturation division is equational and the second reducing. On the other hand if chromosomes pair in the synapsis stage and lie parallel to form double rods, the first division is reducing and the second equational. The chromosomes cannot be distinguished all through the resting period, and whereas I am inclined toward the view that each rod of the double rod represents a chromosome, I have not sufficient direct evidence to be sure that this is the case.

C. The Dichelestid. — Toward the close of the growth period the nucleus undergoes an enormous change. The nucleoli become vacuolated and irregular in outline. The reticulum containing chromatin begins to break up and the nuclear sap stains intensely, probably due to the solution of chromatin. The nucleus becomes irregular in outline, probably due to pressure of yolk. From one to three spheres of protoplasm (Fig. 11) are found in some eggs near the wall of the oviduct, which appear to be abnormal structures. With the breaking up of the reticulum, the chromosomes are formed, but the manner in which this occurs is obscured by the intense staining of the nuclear sap. (This process shows better in *Pandarus.*) In order to see any structures in the nucleus it must be de-stained until it is very faint. The nuclear membrane disappears and the karyoplasm increases in volume and soon some of the chromosomes are distinctly double. The nucleoli disappear suddenly. The karyoplasm fades somewhat and becomes filled with alveoles, some of which are very large (Fig. 12). Each chromosome, of which there are eight (Fig. 13), is surrounded by a sphere of homogeneous protoplasm that stains slightly deeper than the surrounding cytoplasm. Each chromosome is a tetrad and often opens out into a ring constricted at four equidistant points. Two opposite constrictions are deeper than the other two and are to be regarded (from comparison with *Læmargus* and *Pandarus*) as the first divisions formed, and probably divisions between whole chromosomes. When seen on edge the tetrad usually appears dumb-bell shaped. There is some variation in the shape of the chromosomes in early prophases (Fig. 12) but in later stages they appear quite uniform in size and shape (Fig. 14). The spheres of homogeneous protoplasm surrounding the chromosomes fuse into one mass. A colorless area appears around each chromosome (Fig. 13) which makes it appear as though each chromosome was enclosed in a linin sac.

These linin "sacs" are each drawn out in the form of a spindle, and these spindles, lying parallel, form the first maturation spindle. Spindle fibers develop (Figs. 13 and 14) and some of them become attached to the chromosomes. The spindle is elongated, and at each pole the protoplasm stains more intensely resembling the pole plate in protozoön mitosis (Fig. 16). The paler protoplasm forms a sphere around each pole of the spindle, (Fig. 16). From these spheres radiate strands of protoplasm simulating astral rays, but not so dense. The spindle is formed parallel to the flat surfaces of the egg and then begins to rotate to a perpendicular position, at the same time shortening (Fig. 17). In this state it remains until fertilization, and further than this I have not followed it. The behavior of the linin sheath of the chromosome is very peculiar, and it seems to be more conspicuous than in any other instance I know of. The spindle, in general appearance, resembles the first cleavage spindle of *Cyclops* (Häcker).

V. Spermatogenesis.

Læmargus muricatus. (*Preliminary Notice.*)

The spermatogonia are small cells with comparatively large spherical nuclei, and are arranged in single linear series. The nucleus contains chromatin granules in a peripherical linin reticulum. When preparing for mitosis these granules become arranged into looped rows, but I have not ascertained whether these rows form a continuous spireme or not. There is a single large nucleolus. In the last spermatogonic divisions there are 16 chromosomes (twice the number of the spermatocytic divisions). The preliminary spermatocytes are half the size of the spermatogonia when first formed, and are arranged in single linear series. Whether there is a single chain or cord of cells in the testes as in the ovaries, I cannot tell, owing to the many convolutions, but think there are at least several such chains or cords. The regular growth of these causes the testis to be divided into zones. During the growth period the cells grow to the size of the spermatogonia and cannot be distinguished from them save by their position in the testis (growth zone). When preparing for division the cells lose their linear arrangement; the chromatin forms eight double rods which lie close together (synapsis stage). Each double rod becomes ring shaped and the rings contract until only a small lumen is left. An additional pair of constrictions converts the ring into a tetrad. The division forms two secondary spermatocytes, each with eight diads, and another division immediately following forms four spermatids, each with eight chromosomes. The spermatids are grouped in fours. In some of these groups, each of the cells becomes filled with an achromatic substance which presses the chromatin and protoplasm against the cell wall. This substance increases greatly in quantity and becomes more and more like yolk (called Austreibestoff by C. Heider, '79). In very rare cases in some very young spermatids I have seen a small achromatic sphere in the cytoplasm and have found a still smaller sphere in the cytoplasm of some primary spermatocytes. On the other hand the substance in question at first appears to lie within the nucleus and is closely surrounded by chromatin. A plausible explanation might be that the small globule seen in some very young spermatids moved up to and indented the nucleus, there growing until it became larger than the original nucleus yet I have been unable to find steps in such a process, in fact I believe the substance arises in the nucleus.* The cells containing these spheres nourish the spermatozoa, and may be called nutritive cells.

Going back to the spermatids, many groups degenerate, becoming much shrunken, while those that will form spermatozoa collect into larger groups, the cells of which begin to elongate radially. As the spermatids become longer they come to lie nearly parallel. The nucleus elongates into a spindle shaped deeply staining fiber, covered by a thin layer of achromatic substance. The remaining protoplasm fuses with that of adjacent spermatozoa and forms a mass in the center of the group. These groups sometimes lie with one end against a nutritive cell, and when the groups break up the spermatozoa collect around nutritive cells until the cytoplasm of the latter disintegrates and the chromatin collects into rounded masses that float about, leaving only a sphere of a yolk-like substance, the achromatic substance, which has now developed an affinity for plasma stains. As the elements pass down the vas deferens the nutritive spheres lie near its walls and on reaching the spermatophore the nutritive spheres (Austreibekörperchen) form a layer several spheres deep, and become pressed together into polyhedrons. Most of the chromatic spherules form a layer within the nutritive layer but some chromatic spherules caught between the nutritive spheres, become elongate curved bodies that remain for a long time. The spermatozoa fill the center of the vas deferens and lie parallel to one another. In the spermatophores they extend radially from the nutritive walls. In spermatophores that have been attached to the female a long time, the nutritive material has disappeared, leaving a mass resembling evacuated cell walls.

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*Compare formation of "Glanzkörper" from the plasmosomes of Pelomyxa: Goldschmidt, Arch. f. Protistenkunde 5, p. 130.

The same description of the spermatogenesis holds in general for *Pandarus sinuatus*, though there are many minor differences. As regards the behavior of these nutritive bodies I have observed one similar instance, in *Peripatus*, but in *Peripatus* the nutritive bodies are nuclei of degenerating cells. In these copepods the nutritive body appears to form in or in close relation to the nucleus, but too little is known both of the nutritive bodies in *Peripatus* and copepods to suppose them genetically related.

VI. Polarity of the Egg.

The fertilized egg and embryo in all cases in which the egg string extends straight behind the animal and contains a single linear series of eggs, is definitely oriented in relation to the mother. The animal pole of the egg is posterior and the first protoplasmic cell and resulting head end of the embryo is latero-ventral. The chief axis of the egg is manifested in the ovary in the flattening of the egg (being the shortest axis). In any stage in which the primary oöcyte is considerably thicker than the diameter of the nucleus, the latter lies nearer the animal pole. The head end of the embryo coincides with the region in which the first protoplasmic cell is formed, and this is probably determined by the point of entrance of the sperm. The seminal receptical opens into the oviduct by a small orifice which would lead sperm to the egg at or near the position of the future head of the embryo. Cases of rotation of the long axis of the embryo, which sometimes occur, might be due to the spermatozoön getting around the egg before entering.

The first polar body is extruded in a slightly eccentric position on that flat side of the egg directed toward the free end of the egg string. Thus the chief axis of the egg does not exactly coincide with the shortest axis, but is a little inclined toward the anterior end, yet not enough to cause the first polar body to lie in the first cleavage furrow. This is probably due to the great inequality in the first cleavage. Furthermore, the blastopore does not close at a point diametrically opposite to the first polar body, but considerably posterior to such a point. From a study of the literature on gastrulation in crustacea, I am led to believe that the blastopore in the majority of cases in this group closes posterior to the vegetal pole and such a character would be accentuated by flattening of the egg. As the eggs occur in single linear series in an egg string surrounded on all sides by sea water, there is probably nothing in the surroundings that could determine the axes of the egg, and we should regard them as probably determined by the structure of the protoplasm.

VII. SUMMARY.

1. In the maturation of the eggs of parasitic copepods the behavior of the chromosomes in regard to the question of *reduction* is very similar to the same process in the free living copepods, yet I differ from Haecker as to the reducing division, considering the first maturation division most probably to be the reducing division.

2. In the spermatogenesis only a small proportion of the spermatids become spermatozoa. Many spermatids degenerate, others become metamorphosed into peculiar nutritive cells. The protoplasm of the nutritive cells degenerates leaving only a sphere of deutoplasm "Austreibekörperchen," which C. Heider ('79) thought was a glandular secretion.

IX. ABBREVIATIONS.

n = Nucleolus.
p = First polar body.
s = Sphere of protoplasm, probably an abnormal structure
x = Darkly staining cell in yolk.
y = Yolk spherule.

EXPLANATION OF PLATE I.

(*Figs. 1–14, The Dichelestid.*)

FIG. 1. An Oögonium.

FIG. 2. Three consecutive primary oöcytes in the synapsis stage. Optical section of the cord of ovarian cells.

FIG. 3. Early growth stage of same, partial disappearance of cell walls.

FIG. 4. Middle portion of oöcyte containing the nucleus, from longitudinal section of oviduct. Commencement of formation of yolk spherules (*y*). Two nucleoli are seen (*n*).

(*Figs. 5–10, Pandarus sinuatus Verrill.*)

FIG. 5. A little later stage. Nucleus viewed from the animal pole. Two of the eight double rods, or diads, are joined together end to end (to the right), so that it is difficult to distinguish them separately.

FIG. 6. The same in a section through the short axis containing six of the diads. Above at *n*, the nucleolus of another egg is represented to show a later stage in the disintegration of the nucleolus.

FIG. 7. The first maturation spindle.

FIG. 8. Later stage of same, in which it has rotated through almost a right angle and is pushing through the egg membrane to extrude the first polar body.

FIG. 9. Late metaphase of the second polar spindle. The first polar body is represented at *p*. (Constructed from two sections of the series.)

FIG. 10. The same stage from a surface preparation of the egg. The wall of the oviduct was torn off and the eggs allowed to separate in sea water (two hours). In separation the first polar body is pulled out into a stalked structure.

(*Figs. 11–17, The Dichelestid.*)

FIG. 11. Less magnified figure of prophase showing sphere of cytoplasm (*s*) near periphery of egg.

FIG. 12. A later stage. The nuclear sap has faded, it is vacuolated, and around each chromosome stains darker (*f*) than elsewhere. The two chromosomes that are stippled are out of focus. The chromosomes are ring-shaped tetrads.

FIG. 13. The chromosomes are each enclosed in a linin sac, and these sacs have begun to elongate.

FIG. 14. The same or a little later stage. The elongated linin sacs lie parallel, with spindle fibers developed between them. The incipient spindle is formed in dense (dark) protoplasm while this latter is surronnded by looser (paler) protoplasm.

FIG. 15. Equatorial plate, same stage as 14.

FIG. 16. The spindle has elongated perpendicular to the short axis of the egg. The dense protoplasm forms the two poles of the spindle. While the loose protoplasm forms astrosphere-like structures enclosing the poles of the spindle.

FIG. 17. The spindle is half rotated toward the short axis of the egg and has considerably shortened in doing so.

FIG. 18. *Læmargus muricatus* Kröyer. Central portion of the egg containing the first maturation spindle. Metaphase. The spindle fibers do not show in this preparation. Three of the eight chromosomes are in focus, one being seen from the end and appearing smaller than the others.

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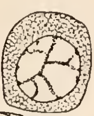

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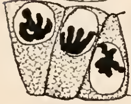

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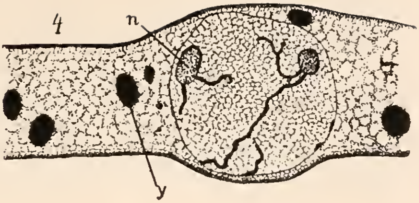

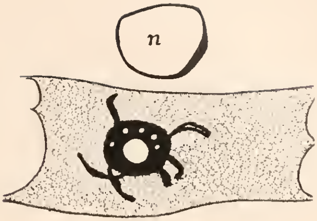

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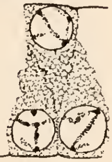

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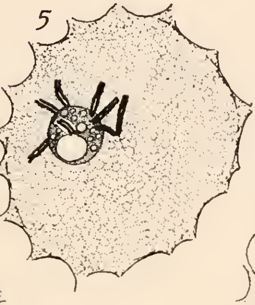

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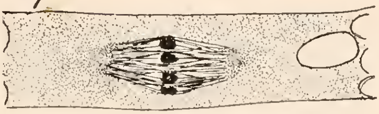

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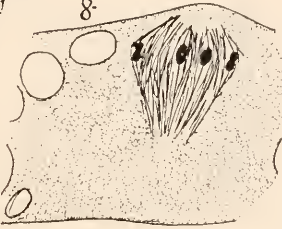

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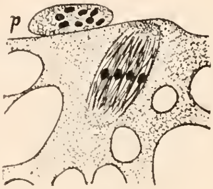

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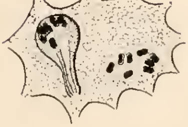

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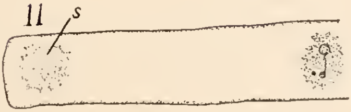

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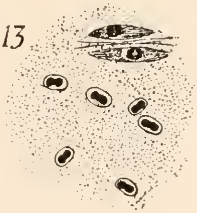

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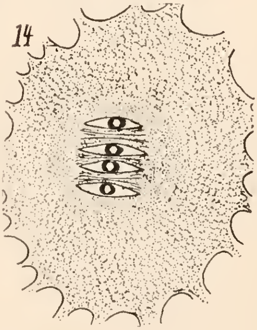

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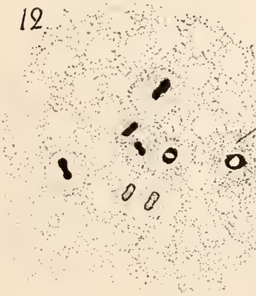

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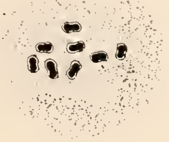

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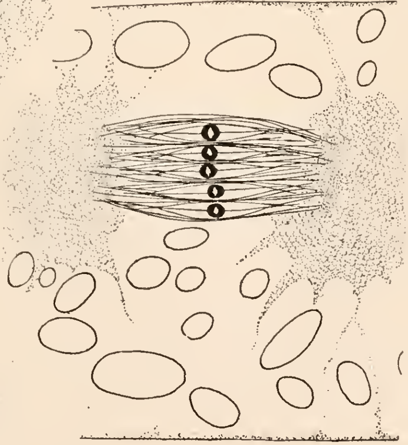

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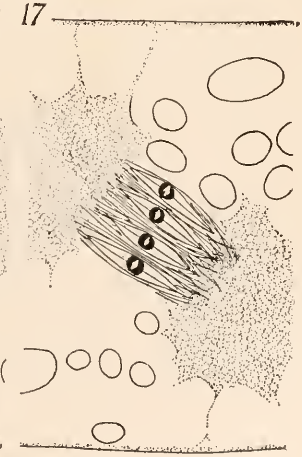

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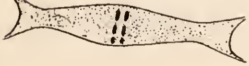

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BIOLOGICAL BULLETIN

ON THE DEVELOPMENT OF PARASITIC COPEPODS.[1]

PART II.

J. F. McCLENDON.

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The egg is covered by a chitinous chorion and the eggs are packed (and much compressed) in a tough chitinous tube (*e. c.*, Text Fig. 1), which is attached to the mother. In order to allow removal from the tube, the eggs must be fixed in fluids which cause them to draw away from the chorion (partly by increasing the osmotic pressure inside the chorion) and which do not make them friable. The only fluids which served contained alcohol and nitric acid. Nitric acid of 5 per cent.–10 per cent. in alcohol of about 30 per cent. served well enough for cell lineage but allowed the chromosomes to swell (partly re-dissolve in water?). The addition of chromic acid (Perenyi's formula) prevented the swelling of the chromosomes and made a good fixitive if manipulated properly. The chromic acid in this mixture is changed to blue chromic oxide, and P. Mayer in the first German Edition of Lee's "Vade Mecum,"[1] says it contains 30 per cent. alcohol, 5 per cent. nitric acid and a little nitric ether and chromic oxide, the last two having no effect in fixation. However, it has been my experience that the chromic oxide was necessary to prevent swelling of the chromosomes. Fischer ('99) says that nucleinic acid is not precipitated by dilute nitric acid, and that its precipitate formed by alcohol is soluble in water. It is possible that the swelling of the chromosomes is due to the solution of nucleinic acid or its compounds in the aqueous staining bath. In addition to its other qualities, nitric acid bleaches the eggs of *Pandarus*, and others that contain pigment, and although it is difficult to wash out (in 70 per cent. alcohol) it was found to be an indispensable ingredient.

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[1] Thesis accepted by the Faculty of the Department of Philosophy of the University of Pennsylvania toward the degree of doctor of philosophy.

I tried various standard fixatives for eggs to be sectioned, and found them little better than Perenyi's fluid; but for whole adults to be sectioned for the ovaries, etc., the latter fluid seemed to be much inferior to some others. It was probably too much diluted by the body fluids.

For staining whole eggs Delifield's hæmatoxylin diluted and acidulated (Conklin's formula) was the most convenient, but for sections various stains were used. In Hermann's safranin-gentian violet, besides the usual differentiations of chromatin, the centrosomes (centrioles) stained red and the archoplasm blue (unless the sequence of stains was reversed). Iron hæmatoxylin gave the sharpest stains for chromosomes.

It is probable that there can be no fixation without some artefacts, and that the differentiation shown by many stains is due to differences in the size and density of particles of protoplasm coagulated by fixation as claimed by some writers. I have paid special attention to the structure of coagulated proteids, and repeated experiments of Fischer and others by producing granular and "curdled" precipitates, and aster-like formations in albumin, and by staining the same. These led me to believe that probably none of the finest structures seen in the fixed protoplasm could be relied upon as representing structures in the living cell, but that such large bodies as chromosomes, spheres, etc., could not be considered artefacts, though their finer structure may be changed.

---

[1] "Grundzüge der Mikroskopischer Tecknik," by Lee and Mayer, Berlin, 1898.

In the cell lineage I have used the quartet system of nomenclature (of Kofoid, '94) as applied by Bigelow to *Lepas* (the only crustacean whose cell lineage has been described beyond the 16 cell stage) to facilitate comparison among crustacea, but do not think this type of cleavage closely related to that of annelids and molluscs, in fact the cleavage of the parasitic copepods does *not* follow a quartet system and I hope no one will be misled by the inappropriate nomenclature.

The cells of the 4 cell stage are designated *a*, *b*, *c*, *d* in a dextral order, *a* being the left anterior cell. An exponent denotes the order of the generation starting with the ovum as the first. A second exponent is used to distinguish a cell from other cells of the same generation and derivation. The odd numbers refer in cases of equatorial division to cells nearer the vegetal pole; of transverse, to cells nearer the anterior end; of longitudinal, to cells nearer the sagittal plane, or in case the cleavage coincides with that plane, the right side. Thus *equatorial* refers to the equator of the chief axis of the egg while *sagittal* and *transverse* to the axes of the embryo that will develop therefrom, but which may be distinguished in the egg as early as the 2 cell stage.

To determine the second exponent of the two daughter cells of any cell division, multiply the second exponent of the mother cell by two and the product is the second exponent of that daughter cell which has an even number for this exponent, and is one greater than the second exponent of the daughter cell which has an odd number for a second exponent.

For cell lineage, whole eggs had to be used, and it was exceedingly difficult to get them out of the egg tube. The best way is to separate the eggs by cutting the tube between them with a sharp "spear head" dissecting needle under the microscope, which increases in difficulty in proportion to the flattening of the eggs. When the eggs are separated the polar bodies are lost and other means of orientation are necessary. In stages before the origin of the primary germ cell it was necessary to lay the eggs on the slide with a determined pole uppermost. The eggs, except when abnormally placed, have the vegetal pole turned toward the mother, and by placing the mother and attached egg strings in cedar oil on a slide under a Zeiss binocular dissecting microscope, it was possible to lay the eggs with vegetal pole up as they were separated, place a cover glass over them to prevent turning, and run balsam under from one side.

Schimkewitz ('96, '99) concluded that pressure was an important factor in determining the form of the cleavage of parasitic copepods.

Pedaschenko ('93, '97, '98) worked for a number of years on the embryology of *Lernæa branchialis* and traced the cell lineage to the 16 cell stage, but was mistaken in the orientation, thinking the first protoplasmic cell to be formed at the animal pole and not distinguishing between the two flat surfaces (dorsal and ventral) of the egg in early stages. He found the germ cells to arise from four cells at the edge of the blastopore and considered two of these to be male and two female. The identity of two of these cells was lost (incorporated in the other two) and the remaining two gave rise to the sex glands. If such were the case, it seems to me that we should expect frequent occurrence of bilateral androgyny (hermaphroditism). The close relation of Lernæa to the forms I studied has made Pedaschenko's work of great service as a hand-book.

Grobben ('79) had long before found the germ cells to arise from four cells of the anterior lip of blastopore of the phyllopod, *Moina.*

C. B. Wilson ('05) includes in his excellent monograph of the Caligidæ, a description of the general embryology of these parasitic copepods.

The only crustacean whose cell lineage has hitherto been carried beyond the 16 cell stage is *Lepas*, as described by Biglow ('02). Pedaschenko pointed out the resemblance between the segmentations of *Lepas* and parasitic copepods and I believe this resemblance is fundamental. The endoblast arises one generation earlier in *Lepas* than in parasitic copepods but this may be due to larger amount of yolk in the latter, which causes a retardation in the segregation of organ-forming substances, and of gastrulation.

Canu ('92) published a paper which I have not seen, which included embryology of copepods. Further consideration of the literature may be found in the text.

I. Cleavage of the Egg.

1. *Læmargus muricatus* Kröyer.

A. First Cleavage. — At the earliest stage I have (Fig. 19) the male and female pronuclei lie side by side at the center of the egg and at the equator of the spindle. At this stage the egg throws out a number of yolk spherules into the space between the egg and the chorion, but the exact nature of this process seems obscure. Each pronucleus contains a nucleolus, and the chromatin is being aggregated into chromosomes. The pronuclei are of the same size and apparently similar in every respect. There is a deeply staining centriole at each pole of the spindle, surrounded by a layer of hyaloplasm that is drawn out into astral rays connected with the surface of the egg, and mantle fibers connected with the pronuclei. The astral rays of one pole are thicker (stronger) than those of the other. Where the mantle fibers come in contact with the nuclear membrane, the latter is pushed in (and partially dissolved?) and finally becomes dissolved, and the mantle fibers become attached to the chromosomes. The spindle thus formed is elongated. The astral rays of one pole shorten more rapidly than those of the other, drawing this pole nearer one edge of the egg than the other. The hyaloplasm is drawn from between the yolk globules and the astral rays increase in thickness. Not only is the hyaloplasm drawn into the astral rays, but small lumps of hyaloplasm adhere to their surfaces and move toward the centrosomes. Thus the spheres increase in size by thickening of the hyaloplasm layer around the centrosome and become surrounded by protoplasm drawn in along the rays. One sphere grows larger than the other and moves to the surface of the egg. Some yolk granules are caught between the astral rays and form a clear space between the sphere and the egg membrane and push the astral rays outward. We thus have a central space almost free from rays, surrounded by an annular area in which the rays are especially aggregated. The center is bulged out and the annular area sunken in by the stress (Fig. 20). Some astral rays connect with those of the other pole, forming "spindle fibers" outside the mantle fibers. The sphere at the surface of the egg soon pulls all the hyaloplasm from between the yolk granules in that half of the egg and the astral rays that pass through the yolk break and are drawn into the sphere. The mantle fibers connected with the outer pole shorten more than those of the opposite pole, and the equatorial plate moves to the plane of the ensuing cell division. The cell division is very unequal, separating a cell containing very little yolk (ab^2) from one containing practically all the yolk (cd^2, Fig. 21).

Going back a little, by the dissolution of the nuclear membranes a good deal of nuclear sap is liberated. This fluid is hard to follow, but I have some slides that seem to show that most of it goes toward the sphere that reaches the surface, and is therefore included in the cell ab^2. The first cleavage plane is parallel to the chief axis, but is very eccentric because of the great inequality of the division. (See the section on orientation of the egg.)

B. Second Cleavage. — After fusion of the chromosomal vesicles in the two cell stages (Fig. 21), the nuclei thus formed remain connected for a short time by interzonal fibers. I have no stages in the division of the centrosome, but soon after this division the two centrosomes are at the ends of a spindle shaped sac or centrodesmus (Fig. 21, small figure to right below). Mantle fibers become attached to the nuclear membrane and the chromosomes gather in that side of the nucleus nearest these points of attachment. From this stage on, the histories of the protoplasmic cell and the yolk cell are different and will be treated separately.

The nuclear membrane of the protoplasmic cell dissolves in the region of attachment of the mantle fibers, which then become attached to the chromosomes. The remainder of the nuclear wall, and the nucleolus dissolve and the chromosomes are arranged in the equatorial plate (Fig. 23), which is at first far removed from the central spindle, but later the central spindle assumes its normal position in the center of the peripheral spindle (Fig. 24). The division divides the cell by a meridional (sagittal) cleavage into equal daughter cells (a^3 and b^3).

In the yolk cell, as the attraction spheres separate they grow in size (Figs. 21–24). The central spindle presses against the nucleus, forming a groove (Fig. 22) which makes it appear as though the nuclues was divided (maternal and paternal elements distinct), but sections show that this division does not pass completely through the nuclues. The nucleus is drawn out to about four times its original length (Fig. 23), one sphere moving faster than the other and reaching the surface of the egg, on the right side of the protoplasmic cell.

This elongated nucleus is bent considerably and suggests that it is being elongated by a force applied internally, and is bent by external resistance, but I think the bending may be due to the unequal pressure of the yolk, and the elongation of the nucleus may be due wholly or in part, to the contraction and separation * of the mantle fibers. The nuclear membrane dissolves, and the equatorial plate is formed (Fig. 24). It is to be noted that whereas the elongated nucleus is bent the fully formed spindle is straight. In Fig. 23 it is seen that the end of the nucleus attached to the peripheral sphere is enlarged and nearer to its sphere than the other end is, probably due to increased tension of the mantle fibers at this end, accompanied by pressure of the yolk on the sides of the nucleus.

On dissolution of the membrane all the nuclear sap goes into the peripheral sphere. A yolk spherule is often caught between the astral rays and the cell wall (Fig. 24, c^3). Protoplasm migrates along the astral rays to the spheres. The division cuts off a small protoplasmic cell from a large cell containing practically all the yolk (d^3). I have not worked out the cell lineage any further in this species, though it appears to be essentially the same as the dichelestid.

---

* By elongation of the central spindle?

2. *The Dichelestid.*

A. First Cleavage. — The earliest stage I have of this is an anaphase of the first cleavage (Fig. 25). It is similar to the same stage in the preceding species save that the centrosomes if they exist at all are larger and less dense, and the sphere reaching the surface collects a considerable mass of cytoplasm around it. The cleavage plane is "meridional" or more correctly, it is perpendicular to the equator of the egg, but owing to the great difference in size of the protoplasmic and yolk cells thus formed, it does not pass through the animal or vegetal pole (Fig. 26).

B. Second Cleavage. — The yolk cell (cd^2) is sometimes retarded in division — in Fig. 26 its nucleus is yet a mass of chromosomal vesicles while that of the protoplasmic cell (cd^2) has reached a late prophase. Already a thickened layer of protoplasm marks the place where c^3 will be cut off.

The protoplasmic cell (ab^2) divides by a meridional (sagittal) furrow into two cells, a^3 and b^3, almost equal in size (Fig. 27). The yolk cell produces an elongated spindle similar to that in the preceding species, one pole of which reaches the surface of the egg to the right (left, when viewed from the vegetal pole) of b^3 (Fig. 27). The protoplasmic cell that is cut off (C^3) often contains a considerable quantity of yolk (Fig. 28). Already a thickened layer of protoplasm (Fig. 33) marks the place where $d^{4.2}$ will be cut off.

In this and the two succeeding cleavages, the poles of the yolk cell spindle are differentiated by the appearance of larger granules on the astral rays of the posterior side of the sphere that is to remain in the yolk (Fig. 27). This probably occurs also in the first cleavage but I have not the right stage to show it. These granules are probably homologous to lumps of cytoplasm on the astral rays of *Læmargus muricatus.*

C. Third Cleavage. — The division of the protoplasmic cells a^3, b^3, and c^3 is equatorial (parallel with the face of the disc) (Fig. 28). The yolk cell gives off a protoplasmic cell, $d^{4.2}$ to the left of a^3. Large granules appear on the astral rays of the posterior side of the sphere left in the yolk. A thickened layer of protoplasm marks the place where $d^{5.2}$ will be cut off.

D. Fourth Cleavage (Figs. 29–30). — In this cleavage the division of the yolk cell ($d^{4.1}$) cuts off a protoplasmic cell $d^{5.2}$ (Fig. 31) to the right of the cap of protoplasmic cells; $d^{4.2}$ divides equatorially, $C^{4.1}$ and $b^{4.1}$ transverselsy; $a^{4.1}$ divides obliquely but the daughter cells ($a^{5.1}$ and $a^{5.2}$, Fig. 31) come to lie one behind the other.

On the dorsal side $a^{4.2}$, $b^{4.2}$, and $C^{4.2}$ divide transversely.

Fig. 30 is an enlarged view of the spindle in the yolk cell, constructed from the two consecutive sections. The astral rays are only partially shown, and, connecting them, the alveolar (or reticular ?) hyaloplasm between the yolk spheres is represented by dotted lines. This is a little later stage than Fig. 29 and the spindle has shortened more. The distinctness of the centrosomes is exaggerated in the figure, in fact it is doubtful whether we deal here with centrosomes, but that the sphere is denser in the center can be shown in some cases with Hermann's safranin-gentian violet stain.

E. Fifth Cleavage (Figs. 31–35). — Of this cleavage I have not enough stages to be sure of the lineage of every cell. There are many disarrangements due to the cells extending over the yolk and slipping on one another, which makes their lineage extremely difficult to follow. In the figures I have divided the derivatives of a, b, c and d by heavy lines, and by comparing Figs. 31 and 32, one can see the great change that has come about.

In this cleavage the yolk cell divides, giving off (near the center of the ventral side) the last protoplasmic cell $d^{6.2}$ (Fig. 32), which is the primary germ cell.

F. Sixth Cleavage. — In the fifth cleavage the divisions were not synchronous, in the sixth cleavage the division of two cells, $d^{6.2}$ (the primary germ cell) and $d^{6.1}$ (the primary entoderm cell) is delayed until some of the other cells are dividing for the eighth time. After cleavage of the majority of the cells (Fig. 33) the blastoderm stretches over the yolk until it has half covered the latter (Fig. 34). During this process some derivatives of d at each side of the egg and at the end of the blastoderm, come to lie under the others and give rise to mesoderm. The yolk cell (entoderm) divides totally by a sagittal furrow (Figs. 34, 35) and the primary germ cell sinks beneath the blastoderm and divides by a sagittal furrow into two cells of unequal size.

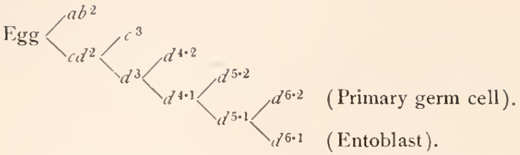

TABLE OF CELL LINEAGE TO 32 CELL STAGE.

II. ON THE NATURE OF THE CLEAVAGE PROCESS.

Since Van Beneden in 1883 put forward the hypothesis that separation of the chromosomes and division of the cell was caused by contraction of the fibers of the karyokinetic figure, the question of the mechanics of mitosis has aroused a great deal of interest. The large size and peculiar form of the spindles in the early cleavage of this egg, make them favorable objects for observations on this point. I have attempted to harmonize observations on the structure of coagulated colloids, and the modern theory of the ultramicroscopic structure of colloids, with the observations on artificially produced asters in colloids, and asters and spindles appearing during mitosis in living cells. A theory of Rhumbler and others as to the mechanics of the formation of asters seems in general to be the only one applicable to the observations I have made, yet I do not believe that this theory is inseparable from the alveolar theory of the structure of protoplasm. Asters can be produced in colloids which we have no reason to believe have the alveolar structure in the strict sense. According to Mann ('06), colloids consist of minute or ultramicroscopic particles suspended in a thin fluid. On congealing (Hardy, Jour. of Physiol., V., 24), these particles by mutual attraction form rows which make up a meshwork (or interalveolar structure ?) giving consistency to the mass. When colloids are coagulated with substances (electrolytes) that act strongly and quickly, the particles are large enough to be seen with the microscope and are at first distributed homogeneously through the fluid, but soon arrange themselves in rows which make up a meshwork ("gerinnselbilder" of Fischer). This passing of a colloid from the "sol" to the "gel" or congealed state may be hastened by addition of a fragment of coagulated colloid to the former, in which case the rows of drops or particles arrange themselves radially around the fragment, and an aster is formed. Fischer ('99) varied this experiment in a number of ways, and one of his experiments. modified slightly, might be tried by every one interested in the subject without much trouble: Spread a layer of egg albumin (which consists chiefly of albumin and a little globulin) on a slide and through a capillary tube introduce a small drop of a fixing solution into the albumin, observing the changes that take place under the microscope. The albumin immediately around the drop is coagulated into a membrane through which the fixing solution diffuses and from which radiations begin to form, giving the whole structure the appearance of an aster with the drop of fixing solution and the membrane around it as the centrosome. If the rays form, as they seem to, by mutual attraction of the drops or particles in the fluid, such rays or rows of drops would exert a pulling force, and if their ends were released should shorten by synæresis into a spherical mass. This may be the nature of the fibers of the karyokinetic figures in the cleavage of these copepods but does not explain the direction of movement of the asters.

III. MESOBLAST.

1. *Nauplius Mesoblast* (*Pandarus sinuatus*, Pl. IV. and V.).

When the cap of the protoplasmic cells has covered about one third of the yolk some of the marginal cells (lip of the blastopore) become differentiated as mesoblast. Of these one or more on the right and left edge will give rise to mesoderm of the first and second antennæ, and one near the middle of the ventral side and distinguished by its large nucleus (Fig. 37) will give rise to the sex or germ cells.

A. The Germ Cells. — This primary germ cell is turned under the rim of the blastopore (Fig. 38) and divides by a sagittal furrow into two (Figs. 39–40), which lie about the center of the ventral side just under the ectoderm. About the time of the closure of the blastopore these two divide by transverse furrows into four (Fig. 41). This group of four cells rotates until one cell is anterior, two lateral and one posterior. (In Fig. 42 the rotation is not quite completed.) But there is considerable variation in the amount of rotation (Figs. 41–48). The four germ cells lie just beneath the ectoderm until the *Metanauplius* stage, when by concentration of the ventral nerve chain, the latter is pushed under them and they rest on top of it (Fig. 56). I have said the germ cells have large nuclei — the nucleus is further characterized by the fact that the chromosomes remain distinct as oval masses just inside the nuclear membrane (Fig. 52). The chromosomes can be counted and are sixteen, just twice the number in the female pronucleus. The cytoplasm is much vacuolated. The germ cells are flattened against each other, and are flattened against the ectoderm in the early stages (Figs. 49, 50). In later stages they detach from the ectoderm, and round up (Figs. 52, 56). And still later (during the *Metanauplius* stage from 24 to 72 hours after hatching of the larva) they separate and pass laterally and upwards into the yolk and two of them come together dorsal to the intestine, and I have not traced them further than the fourth day after the larva hatched, when they were still two in number. Pedaschenko says that two of the four genital cells pass to the right and two (one lateral and one median) to the left. Each pair fuse and, probably by degeneration of one nucleus, becomes a single cell, which finds its way upwards and posteriorly and by division forms the ovary of that side. The fusion of each pair he considers of great significance and the basis of a theory on the origin of the sex of the adult. He believes that one cell of each pair is male and one female and the one whose nucleus persists after fusion of the cytoplasm determines the sex of the animal. His belief that two cells of the four are male and two female is based on comparison with O. Hertwig's account of *Sagitta* in which this condition exists, with difference however in the later history. In *Sagitta* the two female cells give rise to the ovaries (in the anterior part of the animal) and the two male cells give rise to the testes (in the posterior part of the animal).

Sex is said to be determined in some animals by amount of food (of the individual, the parents, or the grandparents) in others by fertilization *vs.* parthenogenesis, in others by dimorphism of egg or spermatozoön, in others by temperature, etc. Pedaschenko proposes an additional factor.

Haecker ('97) found in *Cyclops* the primary germ cell differentiated from the somatic cells at the close of the fourth cleavage or one generation earlier than in the parasitic copepods.

Boveri ('92) in *Ascaris*, and Haecker ('97) in *Cyclops* traced the "Keimbahn" from the first cleavage. In *Ascaris* the visible difference between germ cell and somatic cell was in the chromosomes, in *Cyclops* in the cytoplasm. Early differentiation of the germ cells has been noticed in a large number of animals, but the causal factors in their differentiation are yet unknown. From Boveri's account of *Ascaris*, it seems that the cells of the "Keimbahn" preserve all the characters of the fertilized egg, while the somatic cells lose some characters. Yet the mature ova and spermatozoa of most animals are possibly as highly differentiated as any somatic cell.

In the dichelestid the germ cells have the same origin as in *Pandarus sinuatus* but they differ in appearance. Fig. 57 shows the primary germ cell beginning to be turned under the blastoporal rim. Fig. 58 shows a stage after the division of the germ cell into two cells (of unequal size). If we followed Pedaschenko's theory we might consider the large cell as female and the small cell as male as it is always true that one is larger than the other. The nuclei of the two germ cells lie in their ends that are nearest the free border of the blastoderm (blastopore). These two cells divide into four and the nuclei of two are larger than of the other two, but the cell boundaries between them are extremely difficult to make out.

B. The Mesoblastic Rudiments of the Nauplius Appendages (*Pandarus sinuatus*) arise from cells turned under the rim of the blastopore during epibole. When the cap of protoplasmic cells has covered about one third the yolk (Fig. 38) a few cells are turned under the rim at the extreme right and left, that is to say at the edge of the disc shaped egg. These cells are the mesoblastic elements of the first and second antennæ and divide on each side into two masses (Fig. 40, *an*^{1}, *an*^{2}). The time of this division varies slightly, the elements being sometimes widely separated before closure of the blastopore (Fig. 40) and sometimes close together just after the closure of the blastopore (Fig. 41, *an*^{1}, *an*^{2}). Just before closure of the blastopore, a few cells are turned under its lip on each side (Fig. 40, *md*) and are the mesoblastic rudiments of the mandibles. This completes the rudiments of the nauplius appendages. After the close of the blastopore the post nauplius segments are laid down by teloblastic growth at the posterior end, and the nauplius is pushed (compressed) forward, carrying the rudiments of the second antennæ and mandibles forward (Figs. 47–48), and causing the three pairs of appendages to lie closer together. In stage *D* (Figs. 45–6) the appendages begin to grow out and at the same time the muscle cells elongate into fibers. I think it more profitable to follow these latter backward in development, as it seems doubtful whether they have a single or a double origin. Observe the muscle cells in Fig. 45 elongating radially and attached peripherally to the rudiments of the appendages. In Fig. 44 (Stage *C*) the muscle cells (one shown at *m*) are just beginning to differentiate from the mesoblastic rudiments of the appendages, and two of them have begun to elongate (compare Fig. 50, *m*). The question arises whether all or only some of these muscle cells arose from the mesoblastic rudiments of the appendages.

Just after the closure of the blastopore a few cells similar to these muscle cells are seen considerably removed from the mesoblastic rudiments of the appendages (Fig. 41, *m*). And just before closure of the blastopore minute cells with scarcely any cytoplasm are seen budding off from the ectoderm in this region, (Figs. 40, 49, *x*). There is a slight probability that some of the cells *m* arise by growth of the cells *x* which would be a case of muscle cells arising from ectoderm as in cœlenterata, etc. But small cells with hardly any cytoplasm are found in the yolk at many stages of the embryo (Figs. 44 and 47, *x*) and although I have not closely traced them from cells like *x* in Fig. 40, I think their resemblance in structure indicates a likeness in origin. I think the evidence indicates that all the mesoblast arises from cells turned in from the lip of the blastopore, as is the case in other copepoda, phyllopoda, decapoda and cirripedia.

The muscle cells when first elongated push the ectoderm toward the center and mass it in a sort of structure which somewhat resembles the "dorsal organ" which disintegrates, and the elements of which wander into the yolk. The muscle cells are thus arranged radially just beneath the extremely thin dorsal ectoderm (Fig. 45) but the forward movement of the appendages carries their peripheral ends forward (Figs. 45–48) until they assume a longitudinal direction. Contractile fibrillæ begin to form in the muscle cells in stage *E* (Fig. 47) and the nucleus and undifferentiated cytoplasm is pushed to one side. In the liberated nauplius the muscle fibers run almost the whole length of the animal and show cross striations (Fig. 51). Each appendage then has at least one muscle fiber attached to the anterior and one to the posterior border of its base. Muscle cells that go into the hollow appendages as they grow out, form muscles attached to the bifurcated ends of the appendages (Fig. 51, left side).

The same description in general holds good for the dichelestid and *Læmargus.* In these the mesoderm of the appendages is clearly derived only from marginal cells. In *Læmargus* the ectoderm massed in the middle of the dorsal side by growth of the dorsal muscle fibers forms a more conspicuous "dorsal organ" than in the other species and the elements arising from its disintegration are more numerous.

In relation to the formation of the appendages might be mentioned the segmentation of the nauplius of *Læmargus.* Soon after the closure of the blastopore the embryo is divided by bands of thinner ectoderm into three segments corresponding to the three pairs of nauplius appendages. This segmentation slowly disappears with the development of the nauplius. Other species show it but to a less degree than *Læmargus.* This segmentation might be used as evidence that the nauplius of ancestors of crustacea was segmented or it might be considered as cœnogenetic and associated with the development of the appendages and neuromeres of the nauplius.

2. *Post nauplius mesoblast* (*Pandarus sinuatus*).

At the closure of the blastopore some of the marginal cells are turned in (Fig. 49, *Mp*) and become the mesoblast of the post naupliar segments. These cells are much larger than the surrounding cells (Fig. 41) and form a mass at the posterior end of the animal that is destined to develop mesoblastic somites by teloblastic growth. By rapid division the cells become small and by this time the ectoderm has completely closed over them (Fig. 42). This mass of cells divides in the sagittal plane into two masses (Fig. 44), which begin to grow forward as a pair of broad bands under the neural thickenings of the ectoderm (Figs. 46, 47, *Mp*). From the anterior ends of these bands oval masses are cut off that are the mesoblastic somites (Fig. 48).

IV. Entoblast.

The entoblast is segregated one generation later than in Lepas. In the 32 cell stage the entoblast consists of one cell that contains practically all the yolk and which does not divide until the majority of the cells have completed the seventh cleavage and some are in the eighth. It then forms a very long transverse spindle with the poles inclined anteriorly. The daughter nuclei are widely separated, but in *Pandarus*, *Læmargus* and other Caligidæ the yolk does not segment. The next (second) division occurs about the time the "blastoderm" has covered half the yolk. The spindles extend longitudinally and the poles are inclined outward. Each of the two spindles is shorter than that of the previous division (Figs. 35, 38). The next (third) division occurs about the time of the closure of the blastopore (Fig. 40). There is much variation in the direction and curvature of each of the four spindles, but the daughter nuclei are about equally distributed through the yolk as they are after each division. The fourth division occurs in stage *B* (Fig. 42) and the fifth in stage *C* (Fig. 43).

In *Eudactylina* the yolk segments in the first three cleavages of the entoblast, (forming eight cells) after which the entoblast forms a syncytium. In the dichelestid the yolk divides into four cells and is then transformed into a syncytium. In the remaining species studied a syncytium is formed from the first. This omitting of the cleavage of the yolk is probably not entirely due to the amount of yolk present, which is as great in the dichelestid as in *Læmargus*, but largely due to the extent of compressions of the egg, for it has gone farther in those eggs which are compressed the most. The entoblast nuclei migrate to the surface of the yolk and form the enteron or mid gut, as described by Pedaschenko.

V. Polyspermy.

In *Læmargus muricatus* I have found many eggs into which a number of spermatozoa had entered. In one case the whole egg string was of such eggs; in the other cases only a few such eggs were found in a string. The "development" of these eggs falls under three classes:

1. The ♀ pronucleus and the ♂ pronuclei fuse to form one nucleus in the center of the egg which does not develop further.

2. In the center of the egg a multipolar spindle is formed usually of three principal poles and one minor pole. The resulting division in all observed cases cleaves the egg into three subequal cells, in each of which a bipolar spindle with a very large number of chromosomes is formed. Further development is very irregular.

3. A bipolar spindle with an immense number of chromosomes is formed in the center of the egg. Apart from the number of chromosomes the cleavage approaches the normal type, especially up to the 4 cell stage after which it diverges more and more from the normal type. I am led to believe by certain eggs that show an intermediate stage between a multipolar and a bipolar spindle, that the bipolar spindles in the first cleavage of these eggs are formed out of multipolar spindles.

As all of these eggs were already mounted (by Professor Rynearson) I was not able to observe whether the axes of these polyspermous eggs that approached the normal type in development were the same as in normal eggs. I have not observed whether maturation takes place in polyspermous eggs — the cleavage spindles are very different from normal cleavage spindles, and are very similar to normal maturation spindles. This may be due to a tendency to throw out the excess of chromatin, and in some cases I have found a mass of very small cells extruded from the egg, not always, however, in the position in which polar bodies normally form. There are often many asters in the egg unconnected with chromosomes, and this may account for rounded masses of yolk that are sometimes cut off from the egg.

VII. Relation of Pressure, Etc., to the Type of Cleavage.

When the eggs are released from the oviduct in sea water, they begin slowly to round up and separate one from another. The eggs adhere together so strongly that their tendency to assume a spherical form is greatly impeded, and it always takes several hours for them to round up. The majority of the eggs liberated begin to disintegrate before they proceed very far toward becoming isodiametrical. This is due to their very low surface tension, their cohesion being less than their adhesion for the surface film of sea water or for glass. This is shown by the fact that the eggs tend to stick to the bottom of the glass dish containing the sea water, and when the dish is tilted so that some eggs come in contact with the surface of the water, they quickly spread out over that surface. All these experiments support the direct observation that the oöcyte is surrounded by no other membrane than its surface film.

If eggs are left standing in sea water more than two to four hours their surfaces begin to disintegrate. This is probably caused by partial solution in sea water. The nuclei remain intact after a great deal of the egg has disintegrated. If the eggs are placed in hypotonic solutions they swell, if in hypertonic solutions, they shrink, without any other change that can be observed. I tried solutions of magnesium chloride, ether, and sodium hydroxide, of varying strengths in sea water containing eggs alone oı eggs and sperm but could neither induce parthenogenesis nor fertilization. The spermatozoa are very similar to those of cirripedia, being thread-like and each containing a homogeneous thread of chromatin running the entire length. The sperm of many crustacea are non-motile when examined in sea water or serum, but some of them have been observed to perform movements in the female genital ducts. Cano ('93) saw decapod spermatozoa move lively in the Rec. seminis. It is therefore probable that I did not find the proper stimulus to cause fertilization in sea water. Immediately after fertilization and passage into the egg strings the egg secretes a chitinous chorion that resists all attempts at freeing the eggs so that they will round up, without mutilating them, so I had to resort to looking for eggs that by accident were not flattened in the usual manner. In the Dichelestid the egg at each end of the string is hemispherical in shape, due to the fact that it is pressed on only one side (Fig. 36). In the proximal egg the ventral side is rounded and in the distal egg the dorsal side is rounded. The first protoplasmic cell (*ab*) is cut off at one edge of the hemisphere. The second cleavage results in the formation of three protoplasmic cells (*a*, *b*, *c*) whose centers form the apices of a triangle on the spherical surface at its edge (Fig. 36, *A* and *B*). We should assume that this arrangement is nearer the ancestral type, which was probably a sphere, and that the first three protoplasmic cells being in the equatorial plane (Fig. 27) is due to the pressure. Fig. 36, *A* and *B*, shows a similar arrangement of cells to the same stage in the cleavage of *Lepas* as figured by Biglow, save that in the dichelestid the yolk is much greater in amount and one side of the egg is flat. In both cases *d* (the yolk cell) extends under *a*, *b*, and *c* but in the dichelestid the yolk cell is so large as to push *c* over *b* (in the distal egg).

This altered arrangement of the protoplasmic cells does not seem to affect the normal development of the embryo. The ectoderm grows over the yolk in the usual manner, except that it is stretched more on the rounded side of the egg (Fig. 36, *C*). The four entoderm cells are thicker, and in the distal egg of more volume, than normally and after the entoderm forms a syncytium the nuclei have not exactly their normal arrangement, but when the ensuing nauplius escapes from the egg membrane everything is apparently restored to its normal relation, save that a nauplius developing from a distal egg is larger.

This is contrary to the idea of Schimkewitz, who attributes many abnormalities in parasitic copepod embryos to slight differences in pressure in the egg string; but the eggs he studied had less yolk than those considered in this paper. Differences in pressure in the dichelestid egg result principally in differences in form of the yolk mass. This yolk mass does not, save to very small extent, enter as such into the composition of cells, but is dissolved and used as food by the cells. The protoplasmic cells always being on the surface of the yolk, their relation to the food supply remains unchanged.

Experiments on the effect of unequal compression on cleavage have been made on *Ascaris* eggs by Auerbach ('74); on amphibian eggs by Pflüger ('84), Roux ('85, '93), Born ('93, '94), O. Hertwig ('93); on echinoderm eggs by Driesch ('92, '93, '95), Morgan ('93), Ziegler ('94); on ctenophore eggs by Ziegler ('94); and on *Nereis* eggs by Wilson ('95). These experiments show that if the egg is pressed more on certain sides than on others, as when it is pressed between two plates of glass and forced to assume a flattened shape, that the direction of the cleavage spindles (and consequently cleavage furrows) will be affected. Hertwig formulated the law that the spindle lies in the longest axis of the protoplasmic mass of the cell. This rule probably applies in the majority of cases, but there may be some exceptions, and there are evidently other and unknown factors which enter into the polar differentiation of the cell. Bigelow found in *Lepas* ('02) that the polarity of the egg was not affected by the oval form of the rigid chorion. The principal axis of the egg coincided with the long axis when the chorion was secreted and during the prophase of the first cleavage spindle the egg rotated through a right angle so that the first cleavage spindle was made to coincide with the long axis of the egg determined by the form of the chorion, and the principal or primary axis was perpendicular to it.

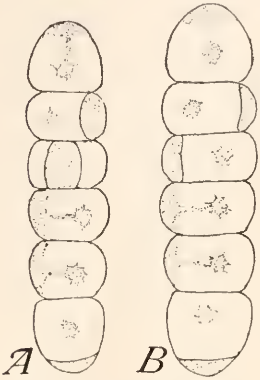

FIG. 4. Egg strings of *Eudactylina nigra* Wilson. The protoplasm is stippled and the yolk white, the distinctness between the two being accentuated. *A*, Living egg string during first cleavage. *B*. The same compressed between the slide and cover glass.

I found a similar condition in *Eudactylina nigra* Wilson. If the egg string of this copepod be placed in sea water under the microscope during the first cleavage stage, the majority of the eggs will have their spindle axes, or in case the division is complete, common cell axis, nearly in the same plane. Often, however, some of these axes are considerably inclined to this plane as is shown in Fig. 4, *A*. If the egg string be pressed between slide and cover glass the above axes will rotate sufficiently to bring them all in a plane midway between slide and cover glass and therefore in the same plane (Fig. 4, *B*). Thus the first cleavage obeys Hertwig's rule whether the compression be applied during or after the metaphase and possibly during the ensuing resting stage.

Hertwig says that if frog eggs are thus compressed normal embryos will develop, although a totally different distribution of nuclei results.

Born found that if a frog's egg were inverted before formation of first cleavage spindle the relative density of protoplasm and yolk would cause streaming movements in the egg, the protoplasm and nucleus rising through the yolk to the upper pole. But it is probable that these streaming movements would be hindered by the astral rays after formation of the spindle.

In the parasitic copepods the direction of many of the spindles is influenced by the pressure, and Hertwig's law applies in most cases. But the peculiar form of cleavage seems well adapted to variations in pressure. The blastoderm lying on the yolk may be compared to a rubber bag divided by lines into polygonal areas. The bag may be pressed into various shapes without altering the mutual relation of adjacent polygons. The only cell whose form is changed very much by pressure is the yolk cell.

I have said that the distal egg (the one at the free end of the egg string) is larger than the others. This is due to the fact that in the oviduct it presented more surface for absorption of nutriment through the wall of the oviduct. While the other eggs present only a thin edge toward the source of food, this last egg of the series presents this edge and one whole flat side in addition. Usually it does not remain flat, but becomes more or less hemispherical on the free side while still in the oviduct.

The cytoplasm of many cells is formed in large part from the substances that escape from the nucleus at the first maturation and early cleavage divisions. Dr. Conklin traced a similar process in gasteropods and in the living eggs of ascidians, and it may be a general phenomenon. In other words, a quantity of chromatin is dissolved and escapes into the cytoplasm in many and perhaps all cell divisions.

In Paramœcium the macronucleus and a large part of the substance of the micronucleus escapes into the cytoplasm at each copulation and may constitute necessary ingredients of the cytoplasm, as copulation is necessary to long continued existence of Paramœcia. It is probably chiefly thus that the heritable qualities residing in the chromosomes are conveyed to the cytoplasm. I do not mean to say that this is the only way the nucleus affects the cytoplasm, for with a few exceptions (*i. e.*, red blood corpuscles of higher animals) cytoplasm not containing a nucleus soon dies, but if the heritable qualities are stored up in the chromatin, part of this chromatin bearing these qualities could be transferred to the cytoplasm more easily during the absence of a nuclear membrane.

After the close of the fifth cleavage (32 cells) the embryo is composed of three types of cells that differ visibly.

1. The primary germ cell.
2. The primary entoblast cell.
3. Thirty cells of the blastoderm all similar in appearance.

The primary germ cell when first separated from the entoderm looks like the other cells of the blastoderm, but during the rest grows larger than its neighbors and is delayed in mitosis. In this character of delayed mitosis it resembles its sister cell (primary entoderm cell). There is nothing characteristic of its position that could cause it to become different from its neighbors, so we must ascribe this difference to the difference in the substances entering into it which in turn may be caused by unequal cleavage.

VII. Summary.

1. My observations on the cell lineage agree in general with those of Pedaschenko (who worked it out to the 16 cell stage) save in regard to the orientation. Pedaschenko used no means to distinguish between the two flat sides of the egg and was mistaken in regard to the location of the animal pole, as I have shown (p. 50). At the fifth cleavage the yolk cell buds off the last protoplasmic cell, which is the primary germ cell. After extrusion of the germ cell (32 cell stage) the yolk cell is purely entoblastic. The segregation of the entoblast takes place one generation later than in *Lepas* (Biglow '02), and the segregation of the germ cells one generation later than in *Cyclops* (Haecker). The delay in the segregation of these two elements is probably due to the large amount of yolk present and the compressed condition of the egg, which cause delay in gastrula-

tion (epibole). It is probable that all the mesoderm arises from cells turned under the lip of the blastopore.

2. The entrance of supernumerary spermatozoa into the egg so greatly disturbs the process of development that the latter is either prevented or so distorted that it never progresses very far, and then in an abnormal manner.

3. The compressed condition of the egg affects the cleavage: (1) by altering the arrangement of the protoplasmic cells, (2) by necessitating increased length of the spindles in the yolk cells, (3) by preventing cleavage of the yolk, and (4) by increasing the surface of the egg and retarding gastrulation (epibole). But it is very improbable that slight alterations in the amount and direction of compression have as great an influence on development as supposed by Schimkewitz. I found nauplii which were apparently normal (save perhaps in size) hatching from hemispherical eggs. As the nauplius hatches it immediately rounds up, and assumes the same form whether it arise from a hemispherical or from a very flat egg.

VIII. EXPLANATION OF PLATES.

ABBREVIATIONS.

an^1 = First antenna.
an^2 = Second antenna.
b = Blastopore.
e = Entoblast cell.
en = Entoblast nucleus.
ec = Ectoderm cell.
f = Deeply staining protoplasm.
g = Germ cell.
m = Muscle cell.
m^2 = Mesoblast of first antenna.
m^3 = Mesoblast of second antenna.
m^4 = Mesoblast of mandible.
m^{5-10} = Mesoblast of post nauplius appendages.
md = Mandible.
mp = Postnauplius mesoblast.
n^1 = Procerebrum.
n^2 = Neuromere of first antenna.
n^3 = Neuromere of second antenna.
n^4 = Neuromere of mandible.
n^{5-10} = Neuromeres of post nauplius segments.
o = Rudiment of mouth.
o^1 = Rudiment of lateral eye.
om = Rudiment of median eye.
x = Darkly staining cell in yolk.

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PLATE II.

(*Figs. 19–24, Læmargus muricatus Kröyer.*)

FIG. 19. First cleavage spindle, prophase.

FIG. 20. First cleavage spindle, metaphase.

FIG. 21. Early prophase of second cleavage. To the right, below, is a highly magnified section of the centrosomes in which the centrodesmus and nucleus of the yolk cell are shown.

FIG. 22. A little later prophase of the same. In the protoplasmic cell the nuclear membrane has begun to dissolve.

FIG. 23. Later prophase showing the elongation of the nucleus of the yolk cell. Viewed from the animal pole.

FIG. 24. Late prophase (the protoplasmic cell is in the metaphase) viewed from the vegetal pole.

Figs. 25–30, The Dichelestid. All eggs viewed from vegetal pole.)

FIG. 25. Anaphase of the first cleavage (fixation poor ?).

FIG. 26. Two cell stage. The protoplasmic cell is in the anaphase of the second cleavage.

FIG. 27. Anaphase of the second cleavage viewed from the vegetal pole (the protoplasmic cell is in the telophase).

FIG. 28. Prophase of third cleavage.

FIG. 29. Late prophase of fourth cleavage $a^{4.2}$, $b^{4.2}$ and $c^{4.2}$ are almost completely hidden by cells lying over them.

FIG. 30. Spindle in the yolk cell, metaphase of fourth cleavage, from two consecutive sections and magnified more highly than Fig. 29. Stained with safranin-gentian-violet. The distinctness of the "centrosomes" is exaggerated. The dark granules on the astral rays of the sphere to the right are lumps of hyaloplasm. The delicate network of hyaloplasm between the yolk spherules is represented by dotted lines but the yolk itself is not shown.

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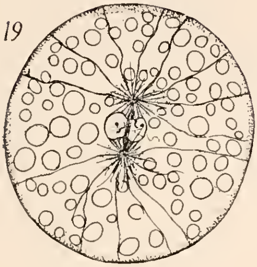

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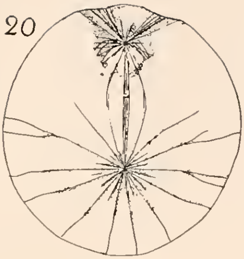

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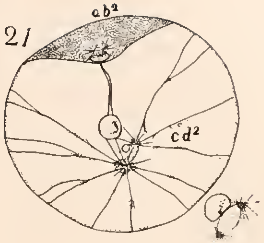

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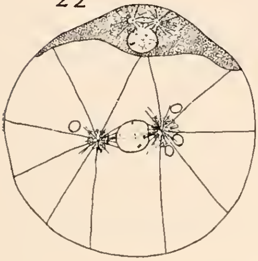

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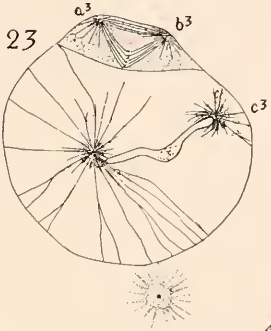

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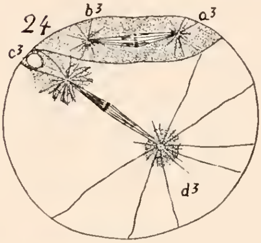

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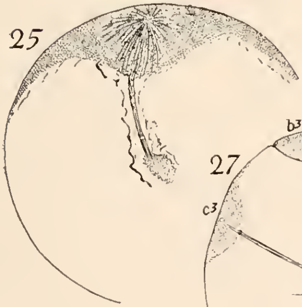

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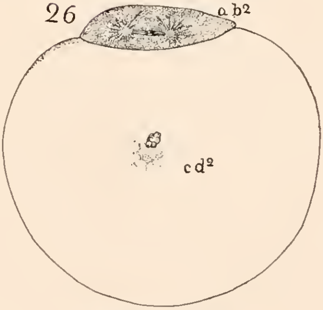

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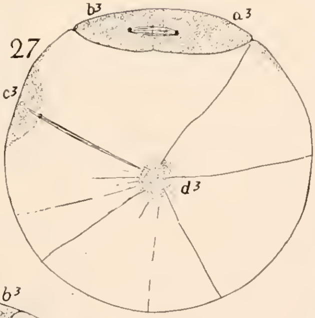

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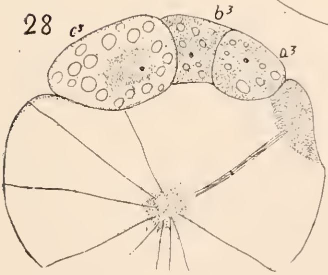

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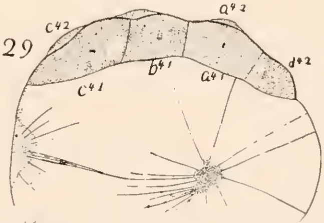

c^{42}
a^{42}
d^{42}
b^{41}
a^{41}
c^{41}

30

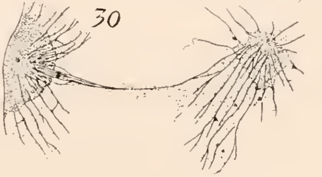


PLATE III.

(In Figs. 31, 32 and 33 the derivatives of *a*, *b*, *c* and *d* are separated by heavy lines.)

FIG. 31. Sixteen cell stage. The cells of the animal-pole-side are shown by dotted lines. $a^{5.1}$, $b^{5.1}$ and $b^{5.2}$ are in the metaphase of the fifth cleavage.

FIG. 32. Thirty two cell stage. The cells of the animal-pole-side are shown by spaced lines.

FIG. 33. Sixty two cell stage. The primary entoblast, $d^{6.1}$, and the primary germ cell, $d^{6.2}$, have not begun the sixth cleavage while $d^{7.10}$, $d^{7.11}$ and $d^{7.12}$ are in the metaphase or anaphase of the seventh.

[*Figs. 34–36, The Dichelestid. Both embryos seen from ventral (vegetal) side.*]

FIG. 34. A later stage than the one shown in Fig. 36, *B*, Plate VI. The primary entoblast cell is dividing. The primary germ cell (*g*) has grown to large size and the blastoderm is beginning to grow over it. At the sides of the figure some mesoblast cells have been turned under the rim of the blastopore (m^{2+3}).

FIG. 35. A later stage than Fig. 34. The entoblast is in the telophase of the second division. The yolk is cut through completely by both divisions of the entoblast. The primary germ cell has divided (*g*) and the blastoderm has grown over it.

FIG. 36. Hemispherical eggs from the ends of egg strings.

A. Dorsal view.

B. Anterior view.

C. Lateral view of stage in which the blastoderm (stippled) has covered half the yolk; the entoblast nuclei are stippled heavily.

(*Figs. 49–56, Pandarus sinuatus.*)

FIG. 49. Sagittal section of gastrula just before the closure of the blastopore (*b*). *p* = first polar body. *mp* = a cell of the post nauplius mesoblast. *x* is taken from another section of the same series and shows a small cell budded off into the yolk from an ectoderm cell. *g* is from one of the same series of sections near the median line, and represents a germ cell in its relation to the ectoderm.

FIG. 50. Part of a median cross-section of an embryo of stage *C* (Fig. 44). The section passes through two germ cells (*g*) a muscle cell (*m*) and two entoblast nuclei. The thickened portion of the ectoderm on the ventral side is the ganglionic rudiment of the second antenna.

FIG. 51. The nauplius just hatched. The ventral aspect is shown in the left, the dorsal in the right half of the figure. The median eye is seen at *om*, and the stomodæum at *o*. The rudiments of the post nauplius ganglia (n^{5-10}) and appendages (m^{5-10}) are clearly differentiated. The entoblasts (*en*) are still scattered through the yolk.

FIG. 52. Section of one of the four germ cells of the nauplius showing the sixteen chromosomes.

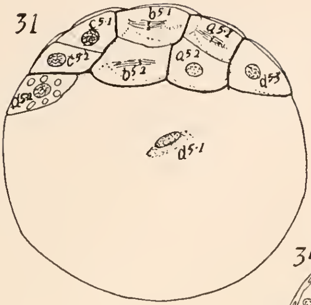
FIG. 53. Enlarged view of section of divided cell from rim of blastopore in Fig. 40. All the chromosomes are not included in the section.

FIG. 54. Prophase from same region.

FIG. 55. Telophase.

FIG. 56. Cross-section through germ cells, ventral ganglia, and ectoderm of nauplius twenty-four hours after hatching.

31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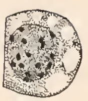
b5.1
a5.1
c5.2
b5.2
a5.2
d5.3
d5.2
d5.1

52



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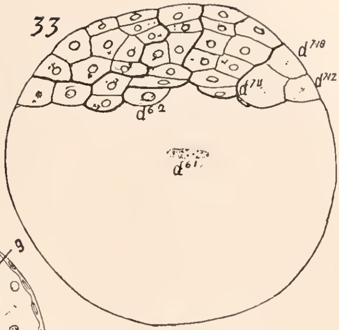
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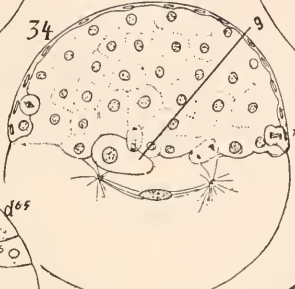
d7.12
d7.11
d6.2
d6.1

34



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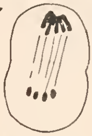

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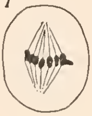

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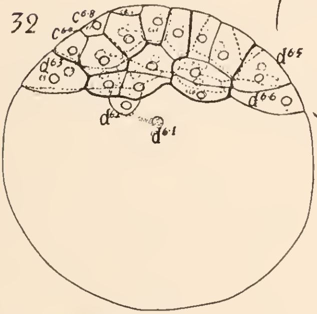
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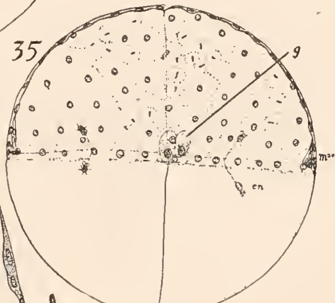
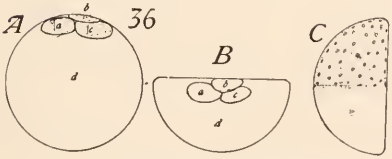
d6.5
d6.3
d6.6
d6.2
d6.1

35



A 36 B C



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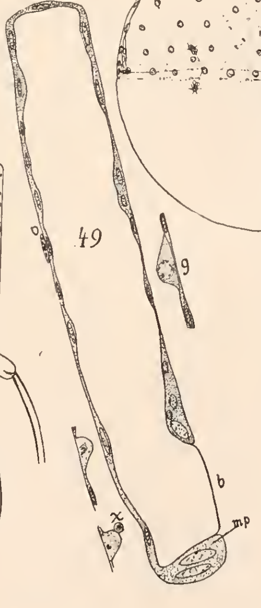

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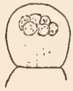

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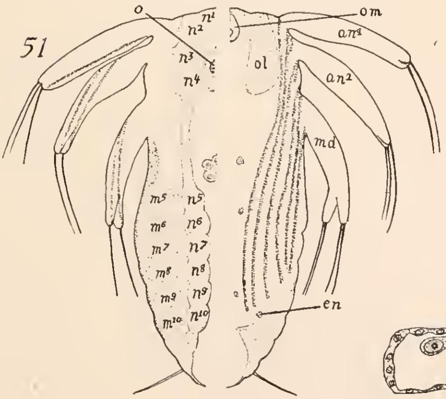
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m5
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n8
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n9
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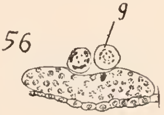
en

56



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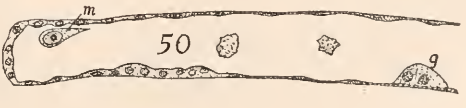


Plate IV.

(*Pandarus sinuatus Verrill. Figs. 37–39, viewed from the vegetal pole.*)

Fig. 37. Shows the metaphase of the first cleavage of the primary ectoblast cell. To the right below is a more highly magnified view of the spindle of the same, in the prophase. *g* = primary germ cell.

Fig. 38. Shows the prophase of the second cleavage of the entoblast. The blastoderm is growing over the primary germ cell (*g*). Some mesoblast cells have been turned under the rim of the blastopore at the sides of the figure.

Fig. 39. About the same stage as Fig. 38, but the entoblast has not begun its second cleavage and the primary germ cell (*g*) is in the metaphase of division.

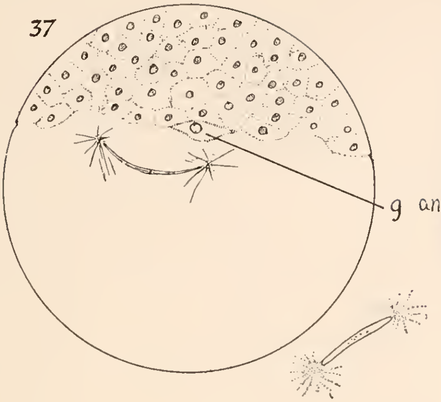
[In Figs. 40–42 half of the dorsal (animal) side of the egg is shown to the left and half of the ventral (vegetal) side to the right.]

Fig. 40. Just before closure of the blastopore (*b*). *X* = small cell budding into the yolk. *An* = mesoblastic rudiment of the first antenna. An^2 = mesoblastic rudiment of second antenna. *Md* = mesoblastic rudiment of the mandible. The germ cells are shown (stippled) beneath the ectoderm — they have separated from one another.

Fig. 41. Stage *A*. Just after closure of the blastopore. The dorsal and ventral ectoderm is omitted save in the anterior and posterior portions. *m*, *m* = mesoblast (muscle?) cells, just beneath the ectoderm. The germ cells have divided.

Fig. 42. Stage *A* a little later than 41. The eight stippled rods are the spindles of the entoblast. The other stippled areas are mesoblast.

37



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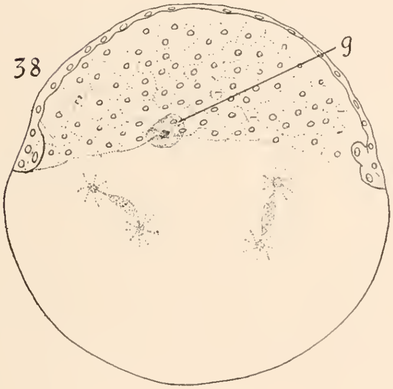

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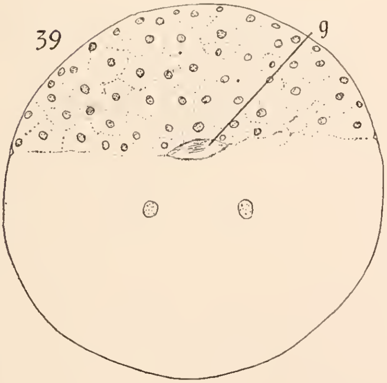

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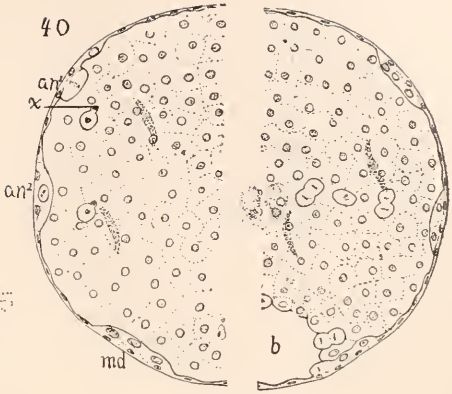
an¹
x
an²
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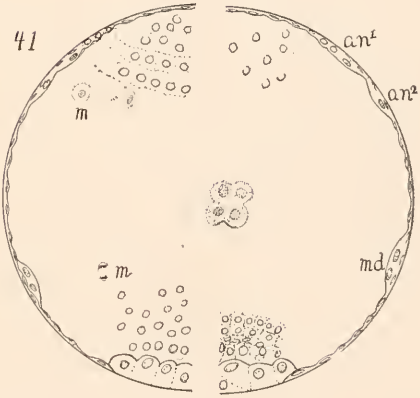
41


an¹
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m
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42

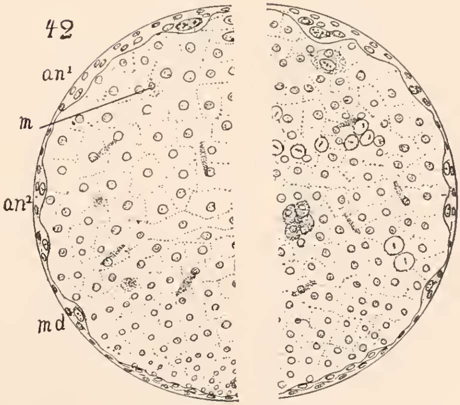


PLATE V.

(*Pandarus sinuatus Verrill.*)

(One half of each figure shows the dorsal, the other half the ventral aspect. In Figs. 43 and 45 the ventral aspect is to the right and in the remaining figures to the left.)

FIG. 43. Stage *B*. The rudiments of the ganglia, n^{1-4}, are shown. The strippled rods are spindles of entoblast nuclei.

FIG. 44. Stage *C*. At *x* are minute (mesoblast?) cells in the yolk. Some muscles in the right side of the figure are beginning to elongate. Over the ganglion of the second antenna is a cluster of mesoblast cells of unknown history.

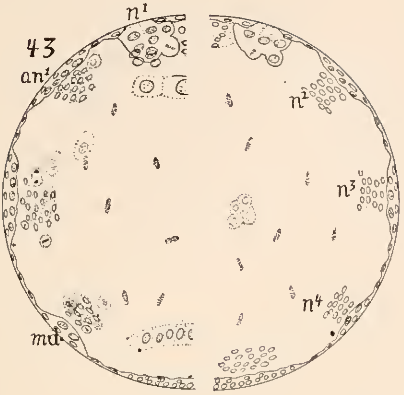
FIG. 45. Stage *D*. The appendages are beginning to bud out. The ganglia have become connected by thickened ectoderm (outlined by a dotted line). The muscle-cells are beginning to elongate radially, and above them the ectoderm cells are elongated in the opposite direction (some of them are represented by dotted outlines, *ec*). The entoblast is not represented, but a few small dark cells are shown in the yolk.

FIG. 46. Stage *D*, later than in Fig. 45. The ectoderm thickening to form the stomadæum is shown at *o*.

FIG. 47. The nauplius a short time before hatching.

FIG. 48. The nauplius just before hatching. In the left half of the figure the ganglia and mesoblastic rudiments of the post nauplius appendages are shown (stippled) in process of formation.

43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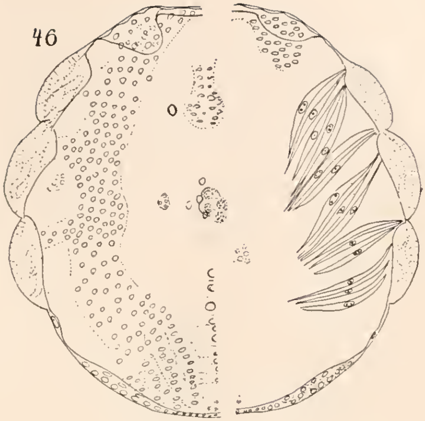
n^2
n^3
n^4
md

46



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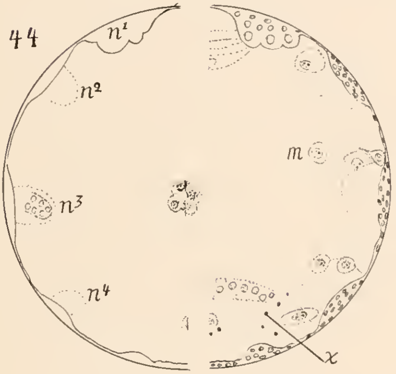
n^1
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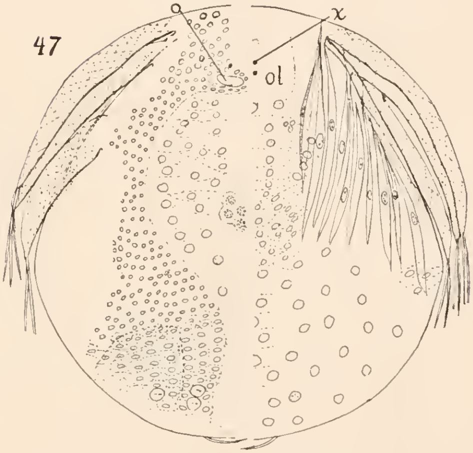
m
n^3
n^4
x

47



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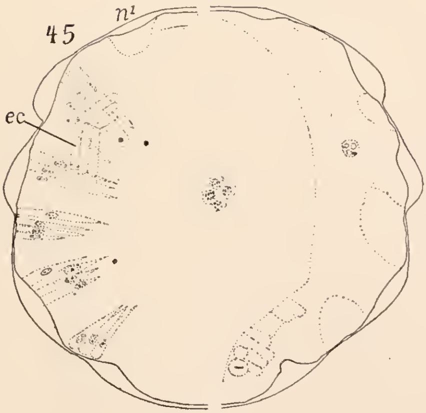

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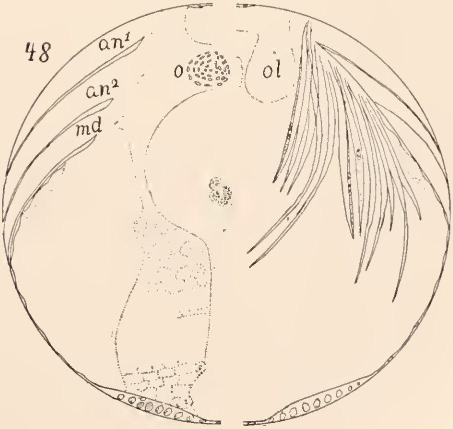
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STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

I. DEVELOPMENT OF THE OVARIES AND OÖGENESIS IN MONIEZIA.

C. M. CHILD.

I. INTRODUCTION.

Some years ago, during an examination of certain abnormalities[1] in *Moniezia*, the apparent infrequency or total absence of any evidence of mitosis even in regions where rapid growth was taking place attracted my attention. Further investigation showed what various authors had already noted, viz., that anything even remotely resembling mitotic divisions was either entirely absent or extremely rare in most of the growing regions. Nevertheless, a rapid multiplication of nuclei was occurring in these regions as could readily be determined merely by the examination of sections of successive proglottids or more exactly by counting nuclei in well-defined corresponding regions of different proglottids. It was soon possible to establish, beyond a doubt, the fact that the characteristic form of nuclear division was amitotic, not mitotic, no single case of mitosis ever having been seen in most parts of the body.

Naturally the next step was to determine whether the development of the germ cells followed the same course. The present and following papers are devoted to a consideration of the results of these investigations.

The species used are *Moniezia expansa* (Blanchard) and *Moniezia planissima* (Stiles and Hassall) both tapeworms of the sheep, which may be obtained in great abundance from the Chicago Stockyards at certain seasons. The work was begun without any preconceived opinions, but as it progressed it became evident that the facts could not be made to agree with the views commonly accepted among cytologists. For this reason the utmost care as regards observations and conclusions has been taken and publication has been delayed from year to year to permit the examination of new material. In 1904 a brief account of amitosis in the early stages was published.[1] Since that time, however, a large amount of new material has been prepared and much of the old reëxamined, but without essentially altering my conclusions.

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[1] Child, "Abnormalities in the Cestode Moniezia Expansa, I. and II.," BIOL. BULL., Vol. I., Nos. 5 and 6, 1900; III., ibid., Vol. III., Nos. 3 and 4, 1902.

Because of the general significance of the data to be described, and in order to forestall possible objections, it has seemed advisable to give in some detail the methods of preparation and procedure. Fresh material was fixed in May or June of four different years. In all, except the first lot of material, the animals were fixed within five minutes after the sheep had been killed. The first material was fixed about two hours after removal from the intestine of the newly killed sheep, but all specimens were alive and apparently in good condition when fixed, and results showed that this material was as satisfactory as that fixed immediately after removal from the host.

A considerable variety of fixing agents was employed in order to discover the best possible fixation and to eliminate the possible effect of particular fixing agents. The fluids used include Hermann's, Chichkoff's, Gilson's, Perenyi's, Merkel's, HNO five per cent., HNO two per cent., HNO two per cent. for one or two minutes followed by Merkel for twenty-four hours or more, aqueous saturated solution of sublimate, sublimate with one per cent. acetic acid and Graf's chrom-oxalic mixture. While certain methods of fixation proved more satisfactory than others the results obtained were essentially identical in all cases so far as the points in question were involved. In the work of the later years Hermann's fluids, chrom-oxalic and aqueous saturated sublimate, were most frequently used as they had been shown to be satisfactory. The chrom-oxalic does not preserve the delicate cytoplasmic structures in the young testes and ovaries as well as does the sublimate, but the nuclear structures are equally clear in both cases.

Various methods of staining were also employed, and while the essential features are visible after almost any fairly good nuclear stain, Heidenhain's iron-hæmatoxylin was most commonly used because of its well-known sharp definition. Undoubtedly this stain is of little or no value for determining differences of chemical composition but it is certainly unsurpassed for bringing out physical differences. Almost the whole body of one worm from the scolex to the region where the uterus contained late cleavage stages was sectioned. From other chains pieces containing three or four proglottids were taken at intervals of from ten to forty proglottids, throughout the body. Pieces separated by thirty or forty or even a larger number of proglottids give stages so near together that nothing is lost, but since it was necessary to make certain that mitosis did not occur periodically in certain regions the intervals between pieces were made much shorter in certain cases. The pieces intervening between those sectioned were numbered and kept and frequently some of them were sectioned later when a larger number of cases of certain stages was needed or some point remained to be settled. Every piece sectioned included at least two complete proglottids and usually more, in the anterior regions often fifteen or twenty. Sections were cut 3–5 μ thick.

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[1] Child, "Amitosis in Moniezia," *Anat. Anz.*, 1904.

New material was sectioned and examined in four different years and each year the old material was reëxamined. Some four hundred camera drawings of nuclei or groups dividing amitotically have been made and in all cases the greatest care has been taken to select those cases which were most clear and convincing. Cases which might possibly be interpreted as amitosis were recorded only when they formed part of a region included in a drawing made for other reasons. The cases recorded by special drawings were usually those which it seemed impossible to interpret in any other way. Many of the cases from which drawings were made were examined by other persons and their interpretation agreed with my own. The observations were all made with a 2 mm. oil immersion lens. With this power some of the divisions may be seen with almost diagrammatic clearness provided fixation and staining are satisfactory.

So far as I am aware I have employed every possible means to establish my observations and to eliminate errors. Amitotic division is not as readily distinguished as mitosis, for there are no visible characteristic stages of preparation and reconstruction and no clearly visible chromosomes and spindles. The dividing nucleus usually does not stain differently from any other, and after division there is in many cases no demonstrative evidence that the nuclei have arisen by division. Certain critical stages in the division must be found, viz., those in which separation is just beginning and the two parts are manifestly connected. Moreover, such stages must be found frequently in order to establish the presumption that anything more than an abnormal or perhaps a degenerative process is involved. My observations have fulfilled these conditions. Thousands of cases of the critical stages have been observed. Cases more or less similar to every case figured in the drawings have been observed repeatedly. As regards a possible failure to recognize mitoses when they occur it should perhaps be said that perfectly distinct and characteristic mitoses do occur at certain times and places and that there is no chance for confusion of these with anything else : chromosomes, spindle, centrosome, equatorial plate, division of the chromosomes, etc., all are visible and instantly recognizable. In fact it has been possible in the germ cells of one species to establish the number of chromosomes with a considerable degree of accuracy. It is very certain that the form of division which I have designated amitosis in these species cannot be interpreted as mitosis indistinctly visible or of peculiar form. All drawings are made from camera drawings and schematization of the actual cases of division and "improvement" of the camera-drawings have been avoided as far as possible.

Many of the figures are schematic in that non-essentials are omitted and simple methods of representation are employed, but every case of amitosis figured is as nearly like the observed case as it was possible to make it after the most careful examination. No attempt has been made to represent the parenchymal substance in which the cells lie in the earlier stages of development, and when, as is often the case, no well-marked area of cytoplasm appears about the nucleus, no cell-boundaries are indicated and the nucleus alone is drawn. As a matter of fact the distinction between cytoplasm and parenchymal substance, at least during the earlier stages, is not nearly as sharp as the figures indicate, for no visible cell membrane is present. For the sake of simplicity, however, the approximate area of the cytoplasm about the nucleus is indicated by a line.

To give a detailed description of the development of the reproductive organs in *Moniezia* is beyond the present purpose. The morphological features concern us only secondarily and will be considered only so far as may be necessary for the understanding of other matters.

II. The Nucleus and the Types of Division.

The nuclei of most of the somatic structures and of the young germ cells do not differ widely in appearance. The nucleus contains a deeply staining " nucleolus " which appears to be at least in large part chromatic in composition and might perhaps more properly be called a karyosome. For the present, however, I prefer to use the term nucleolus. After the usual degree of extraction this is very commonly the only element stained in the nucleus which appears entirely homogenous except for this body. With less extraction other granules are visible scattered here and there through the nucleus, but a distinct reticular structure does not appear. This type of nucleus is shown in most of the figures of earlier stages of ovarian development. In various figures there are cases where the nucleus shows a few small granules in addition to the nucleolus (Figs. 8, *A*, *b*, *d*, *e*, etc., 13, *C*, etc.). Nuclei are frequently found with two nucleoli both of which may be of equal size (Figs. 8, *A*, *f*; 14, *A*, *a*, etc.) or they may be unequal (Figs. 14, *D*; 15, *B*, *a*; 16, *b*, etc.). The question as to whether the two nucleoli always arise by the division of one it has been impossible to settle. Sometimes (Fig. 15, *A*, *a*) a minute nucleolus is found apparently in contact with a much larger one and occasionally (Figs. 7, *c*; 9, *B*) two nucleoli apparently connected by a strand of stained substance are seen. On the other hand, in many cases the two nucleoli of very different size are widely separated (Fig. 8, *A*, *a* and *g*) as if one were arising *de novo*. It seems impossible to decide such questions as this until our methods of study of the cell are greatly improved. From my own observations I should conclude provisionally that both methods of origin exist. It is certain, however, that elongation and division of the nucleolus is not a typical feature of amitosis here. The nucleolus is always spherical or nearly so.

When amitosis occurs each part of the dividing nucleus usually — very probably always — contains a nucleolus. Occasionally it cannot be found but its apparent absence may be due to too great extraction or to loss from the section. It is probable that in *Moniezia* the formation of two nucleoli in a nucleus will be followed sooner or later by division, though division need not necessarily occur at once.

The process of amitosis is simple as far as visible features are concerned, but various apparent modifications occur. In some cases a constriction in the nuclear membrane appears, extending about the whole circumference of the nucleus or limited to one side (Figs. 3, *b*, *c*, *d*, *e*; 5, *a*, *b*; 7, *c*; 13, *A*, *B*., etc.). Frequently there is a faint extension from the deepest part of the constriction partly or wholly across the nucleus (Figs. 3, *b*, *c*; 5, *b*, *c*; 7, *c*, etc.). In other cases the formation of a nuclear plate or membrane across some part of the nucleus takes place before anything more than a very slight constriction appears in the old nuclear membrane (Figs. 7, *f*, *g*; 8, *A*, *e*; 13, *C*; 13, *D*, etc.). In such cases it is possible by careful focusing to follow the new membrane across the whole diameter of the nucleus.

The method of separation of the products of division also varies in accordance with the differences in the earlier stages. The nuclei sometimes separate from one side (Figs. 3, *c*; 5, *c*; 7, *e*; 15, *B*, *b*, etc.), such cases being presumably the result of formation of the constriction from one side. Frequently also the constriction appears to deepen uniformly about the whole circumference (Fig. 3, *d*, *e*, etc.), separation being completed at or near the middle. In those cases where a distinct nuclear plate or partition forms across the whole nucleus separation seems to occur simultaneously or nearly so over the whole surface (Figs. 8, *A*, *c*, *i*; 9, *A*, *b*, etc.). These cases are perhaps the most demonstrative of all, for the flattened surfaces of the two nuclei and occasionally their contact at one margin (Figs. 8, *A*, *c*; 9, *A*, *b*) leave no room for doubt that division has actually occurred. Occasionally the margins of the two nuclei are the last portions to separate (Fig. 16, *a*). The flattened surfaces are undoubtedly soon lost after division. Some of the cases of separation from one side show very clearly that the separated parts of the surface begin to become convex before separation is completed (Fig. 5, *c*).

The division of the cytoplasm also varies to some extent, but is more difficult to observe since the cytoplasm is usually without any sharply defined boundary. In some cases a constriction of the cytoplasm follows the constriction of the nucleus (Figs. 3, *a*; 7, *f*, *i*; 9, *A*, *d*; 29, *b*, etc.), and in others the nuclear division may be completed before the cytoplasm shows any trace of constriction. (Figs. 7, *d*, *g*; 8, *A*, *c*, *e*, *i*; 11, *A*, *b*; 14, *A*, *b*, etc.). Occasionally nuclear and cytoplasmic division are apparently almost simultaneous (Figs. 7, *h*, *i*; 8, *A*, *h*, etc.). A few cases have been noted where the division of the cytoplasm is "endogenous." Such a case is shown in Fig. 11, *A*, *a*; and another in Fig. 21, *a*. Such cases as Figs. 2, *a*; 5, *d*, and *29*, *a*, seem to indicate that a "cell-plate" may sometimes be formed across the cytoplasm, though after the appearance of a cell-plate it is of course impossible to be absolutely certain that the two cells are the product of a division. But the fact that the nuclei with their surrounding areas of cytoplasm are usually isolated renders it probable that such cases represent division.

Not infrequently one part of the nucleus stains more deeply than the other. In such cases the stain is uniformly distributed in each part but the boundary line between the darker and lighter portion is sharp. Figs. 7, *b*, 14, *B*, *a*, and 16, *c*, show cases of this kind. This difference in staining is of some importance as indicating that there are differences of some sort in the two regions and probably also that the two are functioning more or less independently.

Occasionally a case of what appears to be an "endogenous" division is found (Fig. 13, *E*). In such cases the nuclear plate or new membrane does not appear as a simple partition but two distinct membranes more or less convex toward each other are formed, while the old nuclear membrane appears to surround the whole. Some cases of this sort of almost diagrammatic clearness have been observed and in one of the turbellaria, a form with much larger nuclei, I have recently seen something similar and am therefore inclined to believe that cases of this sort actually do occur. In later stages the old membrane seems to disappear leaving two separate nuclei.

The occurrence of amitosis is probably to be regarded as the result of the establishment of more or less independent functional regions in different parts of the nucleus and the consequent formation of a membrane about each of these. The details of the process must of course differ according to conditions so that many different forms of amitosis may occur, all due primarily to the same factors. The "endogenous" method of division is not perhaps so widely different from the others as might appear. The old nucleus is so large or the new "functional" nuclei so small that parts of the old nucleus are left out when the new membranes are formed. Ordinarily the new membranes are formed in direct contact, here they are merely formed separately.

As regards the process of mitosis but little need be said here. The maturation mitoses will be described later but the tissue mitoses and those in the germ mother-cells differ more or less from these. In preparation for mitosis the nucleus stains more deeply and traces of a spireme are sometimes seen but the preparation first becomes readily recognizable when the chromosomes are formed (Fig. 8, *A*). The largest number of chromosomes counted is fourteen but accuracy is out of the question here. Fig. 8, *B*, shows a case where twelve were clearly seen. A spindle in metaphase is seen in Fig. 8, *A* ; it is usually possible to distinguish dark bodies at the poles but much depends on the degree of extraction ; the spindle fibers are very delicate and astral radiations are not certainly distinguishable. A later stage is seen in *a*, Fig. 5. Any stage of the division from the formation of the chromosomes to and including the late anaphase is as readily recognizable in the sections as in the figures.

The occurrence of these two forms of division side by side is an indubitable fact, but further data will be given in following papers. There can be little doubt that each form of division is a reaction to special conditions. Certain observations of my own indicate that amitosis occurs more frequently in very rapid and mitosis more frequently in slower growth. But until other data are described theoretical considerations are out of place.

III. THE EARLY STAGES OF THE FEMALE REPRODUCTIVE ORGANS.

The ovary itself does not appear in the earliest stages of development of the female organs. It is in fact formed only after a considerable portion of the ducts has differentiated. The earliest visible stage in the development of the female organs is the increase in the number of nuclei in a region immediately adjoining the longitudinal nephridial canals. Viewed from the surface it appears as in Fig. 1, *A*. A somewhat later stage is shown in Fig. 1, *B*. The nuclei are more closely packed together here than elsewhere consequently the region stains more deeply. This region differentiates later on into the middle region of the reproductive ducts. Sections through this region at this stage show a number of nuclei often surrounded by more or less cytoplasm without definite boundary. These nuclei are indistinguishable from the other parenchymal nuclei except that many of them are smaller. Parenchymal fibers can often be traced from the cells and the region is not marked off in any way from the parenchyma.

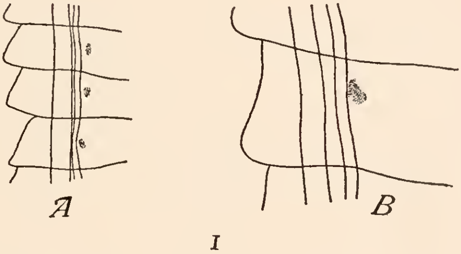


FIG. 1, *A*, 1, *B*.

But the most interesting point forpresent purposes is the apparent absence of mitosis in these regions. Although the writer has examined hundreds of sections of these early stages he has never seen a single case of mitosis. Yet it is very evident that an exceedingly rapid multiplication of nuclei is taking place, for the size of the area and the number of nuclei increases rapidly with increasing distance from the scolex. Figs. 2 and 3 show small portions of this proliferating region. Fig. 2 is from *M. planissima*, Fig. 3 from *M. expansa.* The cytoplasm about the nucleus is often so slight in amount as to be almost invisible. All the cells lie free in the parenchyma. A number of nuclei in both figures show various stages of amitosis and others have appar-

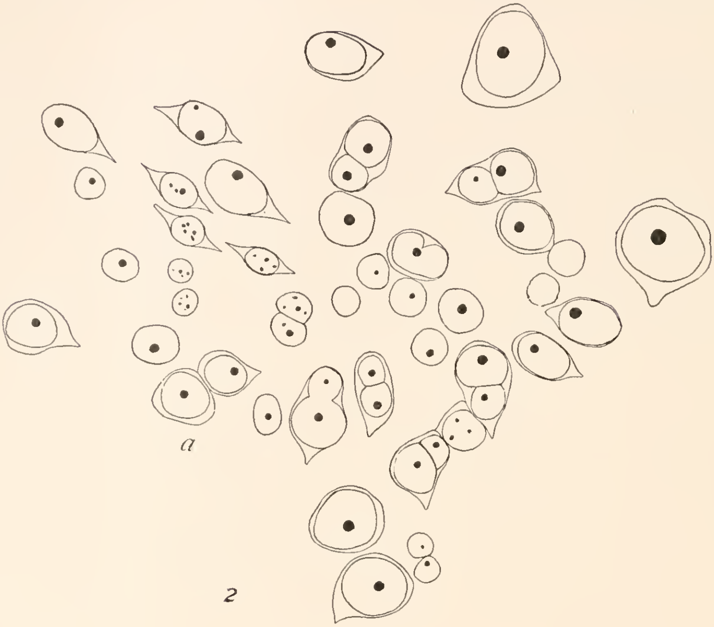


FIG. 2.

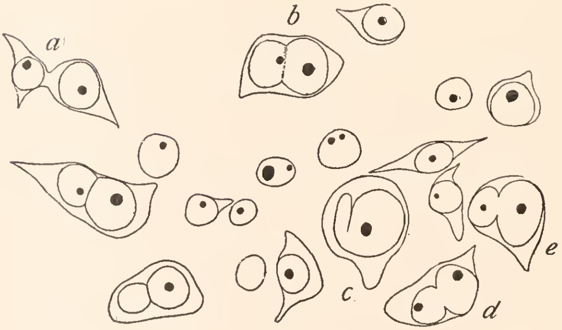


FIG. 3.

ently only recently separated. From this stage up to the period when the ducts begin to assume definite form the appearance of these regions is much the same. The course of development of this area certainly favors the view that the proliferation is due to some localized stimulus or condition rather than to anything inherent in the cells themselves. In the central portions of the area the nuclei have divided so rapidly that they are very small ; nearer the periphery they are larger and about the outside of the area are nuclei of the same size as other parenchymal muclei. Divisions likewise decrease in frequency from the center toward the periphery. Fig. 2 shows this difference to some extent. The nuclei near the center of the figure which represents approximately the center of the proliferating area are much smaller than those about the periphery. The two large nuclei in the upper right corner are about the size of typical parenchymal nuclei. Fig. 3 is a smaller area from a somewhat later stage and entirely within the proliferating region.

The later development of the ducts will be considered more fully in connection with other somatic structures. It is typically a process of continued amitotic division although in some individuals an occasional case of typical mitosis occurs.

IV. The Formation of the Ovary.

From the region of its first appearance near the nephridial canals (Fig. 1) the proliferating area gradually extends somewhat toward the median plane and toward one surface of the proglottid known as the ventral surface. So far as can be determined there is no appreciable migration of cells through the parenchyma ; each part seems to be formed *in situ*, the stimulus to proliferation continually involving more of the parenchymal nuclei and extending in a more or less definite direction.

As the inner and ventral end of the proliferating area approaches the inner layer of circular muscles it spreads out into a flattened somewhat disc-like area exactly as if it had encountered resistance to its growth in the original direction and so had begun to spread out in other directions. Fig. 4 shows a stage soon after the disc-like flattened terminal region has appeared. This disc-like terminal portion indicated by *o* is the ovary. In connection with it and indicated diagramatically in the figure is the oviduct. The outer portion of the female duct, *i. e.*, that leading from the region of the nephridial canals, is still in an early stage of development and the genital opening has not yet appeared. This sequence in the formation of parts is rather peculiar; the first portion to appear becomes the middle region of the ducts while inner and outer terminal portions, including the ovary appear considerably later.

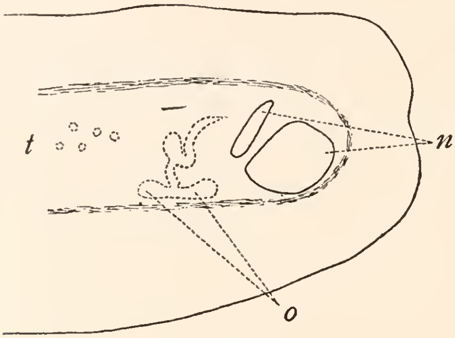


Fig. 4.

In its earliest stages the ovary does not differ very widely in appearance from the early stages of the ducts. It consists merely of a number of nuclei in the parenchyma surrounded by more or less cytoplasm and undergoing frequent amitotic divisions. But the divisions seem to be somewhat less frequent here than in the regions of the ducts and the nuclei never become so reduced in size. But one difference between the ovary and the early stages of duct-formation exists: viz., the occasional occurrence of a case of mitosis in the ovary. Fig. 5 illustrates very clearly the occurrence side by side of the two forms of division. It represents a portion of a longitudinal section through the inner end of the oviduct and the ovarian region. The smaller cells (*od*) on the left in the upper part of the figure with elongated cytoplasmic areas represent the terminal portions of the oviduct and the region between these and the muscles, which are represented by small circles, the ovarian region. In this section only a few of the cells involved in the formation of the ovary appear; they are

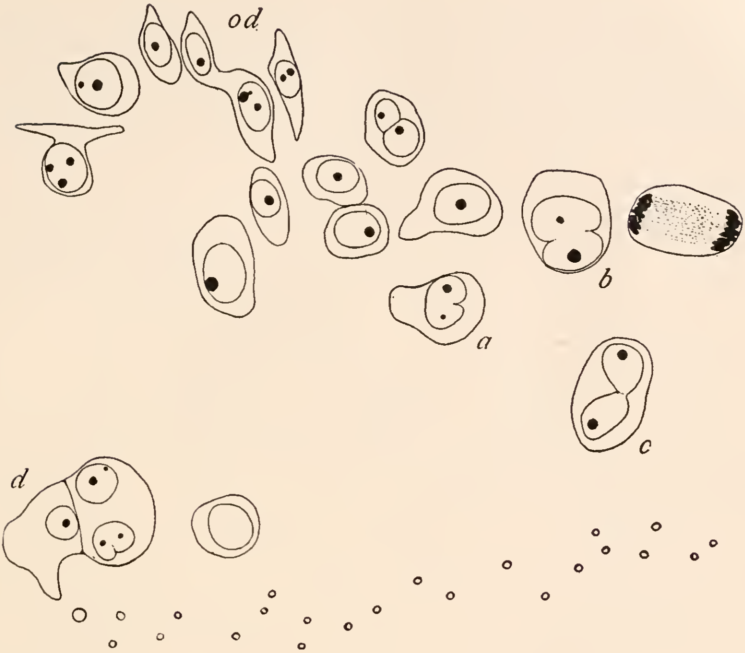


FIG. 5.

mostly in other sections. On the right is a typical case of mitosis in late anaphase ; this is the earliest case of mitosis seen in the ovary ; *c* is a very distinct case of amitosis, *b* is a second case. The smaller nuclei also show two cases of amitosis. Whenever mitosis occurs in the developing ovary other nuclei dividing amitotically are found near it.

V. GROWTH OF THE OVARY.

The further development of the ovary consists in increase in size of the proliferating area and a little later the outgrowth from its margins of finger-like follicles which elongate and give the ovary its characteristic form. A follicular membrane differentiates, apparently from the cytoplasm of the cells about the periphery of the proliferating region, thus separating the ovary from the general parenchyma. Fig. 6 shows a stage in the later development of the ovary in which the follicles have attained almost complete development.

The posterior portion of the disc-like proliferating area which terminates the oviduct at the stage of Fig. 4 is indistinguishable from other parts in the earlier stages but in the later stages shows smaller cells and smaller follicles than the ovary and forms the vitellarium. It is not shown in Fig. 6.

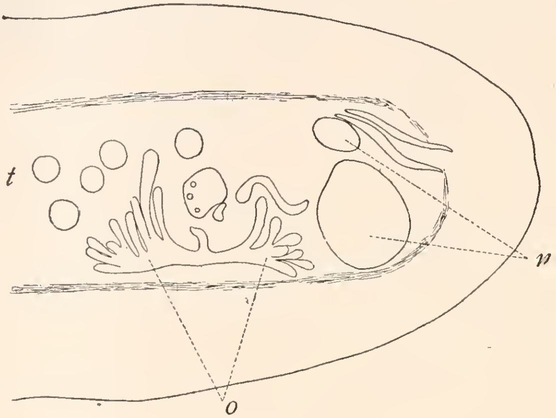


FIG. 6.

After the development of the ovary is completed nuclear division ceases and the mother cells enter upon the first stage of their development as ova. As long as division continues, however, it is predominantly amitotic. The following figures taken from various stages between the formation of the ovary and the completion of its development in both species will show very clearly the prevalence of this process.

During the growth of the ovary the parenchymal substance remaining within the ovary gradually disappears. In many cases, especially after chrom-oxalic, the cytoplasmic areas about the nuclei are indistinct. The cytoplasm is usually not sharply marked off from the parenchymal substance still present so that the whole often appears as a syncytium. After sublimate fixation the cytoplasmic regions appear more distinct. Most of the figures are drawn from sublimate preparations.

Fig. 7 is from a stage slightly later than Fig. 5, before the separate follicles have appeared and before an ovarian membrane has formed. The width of the figure represents the whole width of the young ovary. In the five nuclei *d*, *e*, *f*, *g*, *i* there can be no doubt regarding the occurrence of amitosis. Each one of these nuclei was examined at all levels and in such cases as *f* and *g* the membrane can be followed through the whole nucleus. The case indicated by *b* is one of those often found where the two parts of the nucleus stain differently. At *f* the two nuclei have apparently recently separated for they are nearly hemispherical in form, their flattened surfaces are parallel and closely approximated and are not visibly covered by cytoplasm.

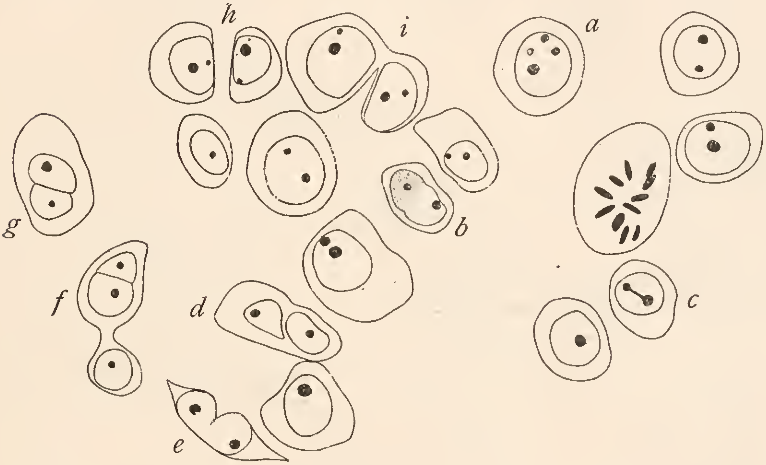


FIG. 7.

The two nuclei at *i* are apparently also the products of a recent division. The cytoplasm is still continuous on the upper side and the nuclei are flattened on opposing faces. The figure also shows one case of mitosis.

Fig. 8, *A*, is from the same proglottid as Fig. 7. At this stage muscle fibres are still visible passing directly through the ovary and are indicated in the figure. It contains six cases of mitosis, the largest number observed in any area of similar size. But in this section are also two very clear cases of amitosis, (*c* and *i*). The cases *b*, *e*, *h* and *j* are also undoubtedly amitoses and several other nuclei in the figure are probably also dividing amitotically. The number of mitoses in this section is of interest since it exceeds so greatly the number seen in any other similar case. Commonly section after section may be examined without seeing a case of mitosis or occasionally one or two may be found. In the ovary shown in Fig. 8, *A*, however, several consecutive sections showed frequent mitoses particularly in one region of the ovary. Amitosis was common everywhere as usual. Discussion as to possible determining conditions is postponed for the present. Fig. 8, *B*, is a case of mitosis in which twelve chromosomes were clearly visible — probably not the whole number. It was usually impossible to determine with accuracy the number of chromosomes in these divisions.

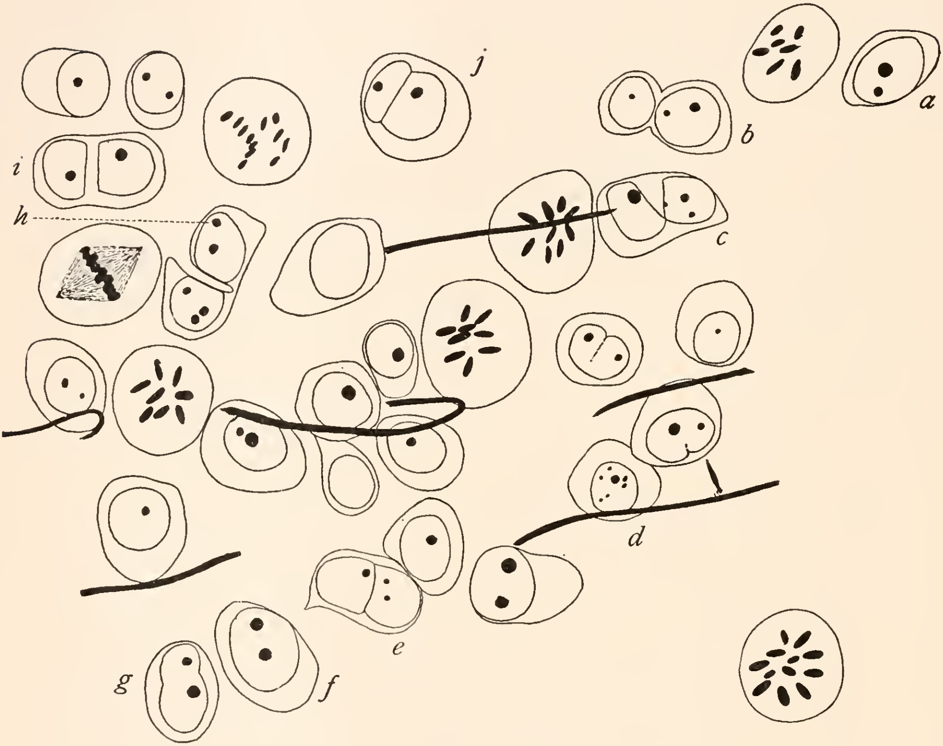


Fig. 8, *A*, 8, *B*.

Fig. 9, *A*, is also taken from the same stage and across the whole width of the ovary near its base. At least four perfectly clear cases of amitosis (*a*, *b*, *c*, *d*) are present besides two other

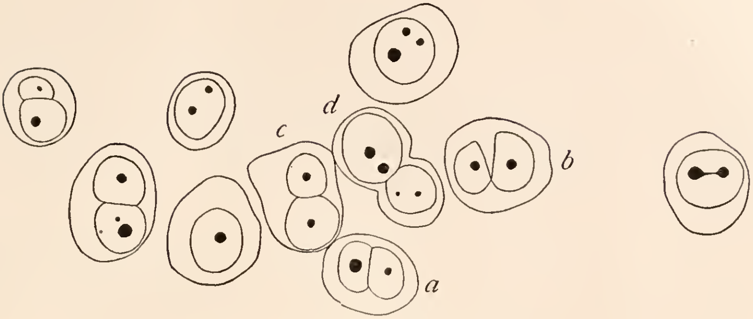
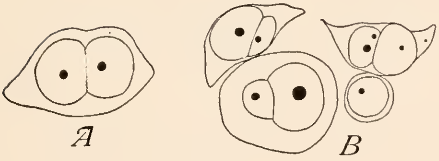


Fig. 9, *A*, 9, *B*.

doubtful cases. Fig. 9, *B*, is a cell from the same ovary with two nucleoli apparently connected.

The Figs. 7–9 are taken from *M. expansa*. Since the course of development is identical in both species so far as can be observed, it is not necessary to duplicate all stages in two species, but a few typical cells and cell-groups from the ovary of *M. planissima* are figured. Fig. 10, *A*, and 10, *B*, both show characteristic cases of amitosis in the earliest stages of ovarian development. Fig. 11, *A*, shows two cases and Fig. 11, *B*, one. The case *a* in Fig. 11, *A*, represents a rather interesting stage



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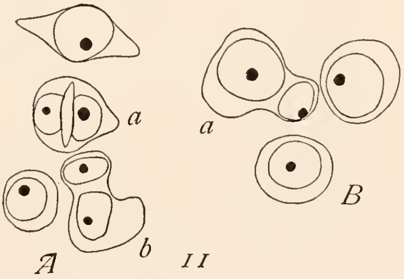

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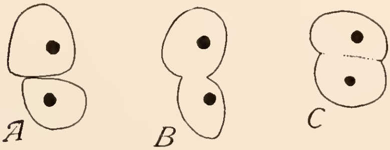

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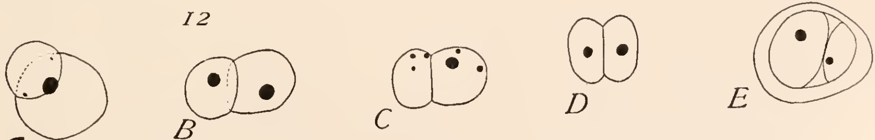


13

Figs. 10, *A*, 10, *B*, 11, *A*, 11, *B*. Fig. 12, *A*–12, *C*. Fig. 13, *A*–13, *E*.

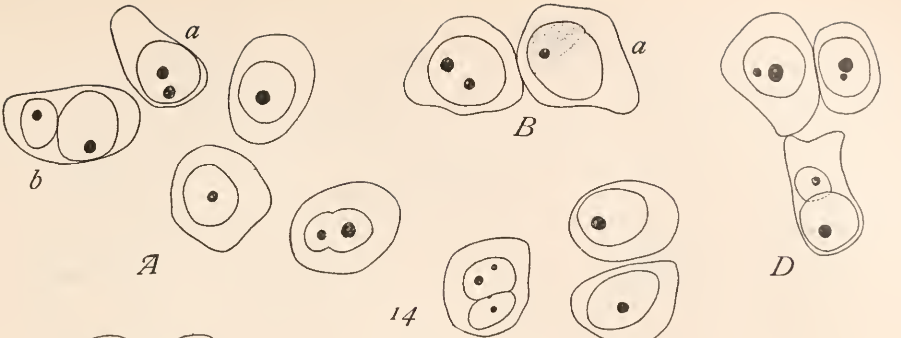
and one that possibly does not always occur. The nuclei have separated and between them there is a lenticular space apparently empty, but probably containing fluid in the living condition. This space is apparently still intracellular for the cytoplasm is clearly continuous across its ends. In most cases of amitosis the two nuclei separate at one end first so that no such space is formed.

In Fig. 12, *A* and *B*, the same nucleus is shown at different levels, *A* being the upper portion and *B* the lower. *C* is probably an early stage of amitosis. Both are from slightly later stages of development than Fig. 11.

Cases from a still later stage are shown in Fig. 13, *A–E*. The chain from which Figs. 10–13 were taken showed fewer mitoses in development of all organs than any other examined. Mitosis was scarcely ever seen in the ovary. That individual differences do exist in this respect can scarcely be doubted.

After the individual follicles begin to form, the divisions seem to be more frequent in them, and especially near their tips, than in the central portions of the ovary. Fig. 14, *A–D*, show groups with characteristic amitoses from the developing follicles of *M. expansa*. Fig. 15, *A*, and 15, *B*, are similar, the latter showing a few nuclei from the extreme tip of the young follicle not yet enclosed by a follicular membrane. In Fig. 16 the terminal portion of another follicle is shown with three cases of amitosis.

An early stage in the development of the follicles in *M. planissima* is shown in Fig. 17. The numerous cases of amitosis are clearly visible. At *a* in the left follicle is what appeared to be a group of small nuclei. It may be a multiple amitosis or may possibly represent a stage in reconstitution after mitotic division. The large nuclei in two of the follicles are frequently found along the axes of developing follicles. One of them (*b*) is apparently dividing into three parts. The nuclear divisions which have been described thus far are in reality the oögonial divisions. The amitotic divisions certainly constitute a normal feature in the history of the ova, as there is no evidence that the nuclei which have divided mitotically have a different fate from the others. The relative frequency of mitoses varies not only in different chains



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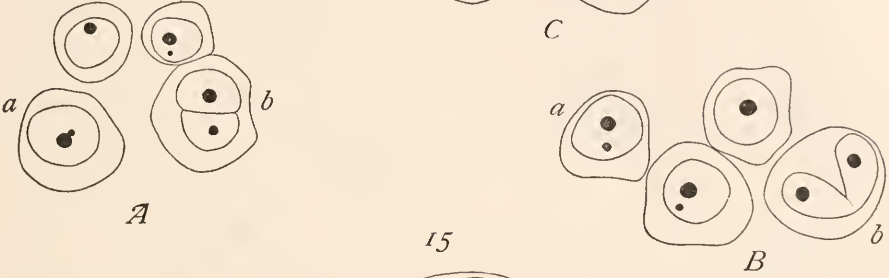

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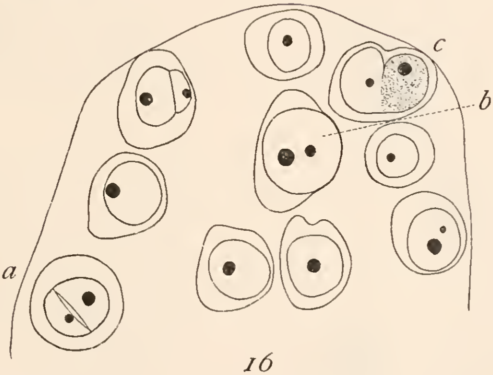

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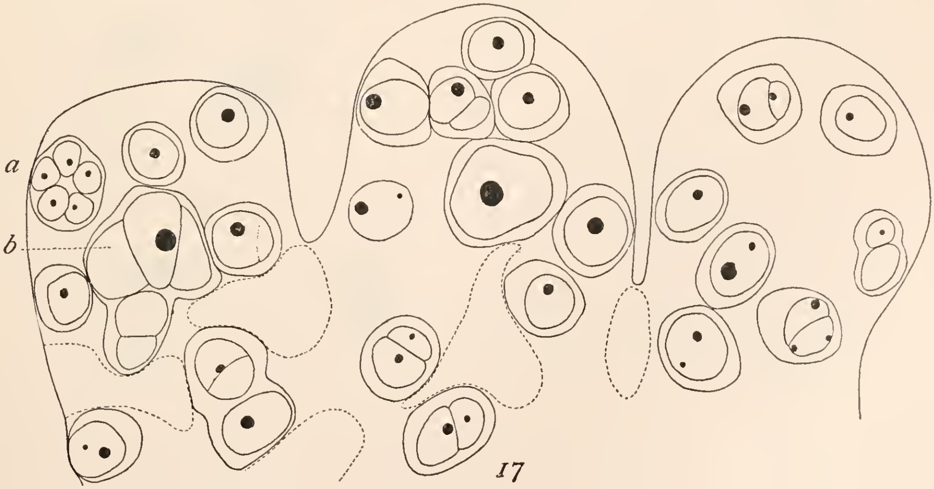

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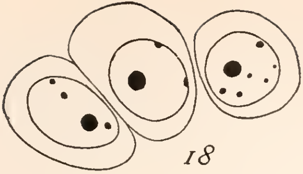
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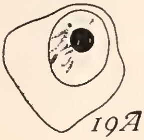
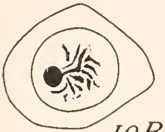

19A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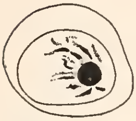
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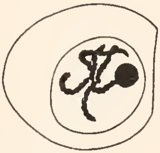
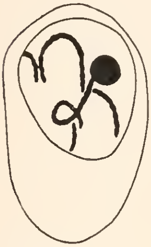

20A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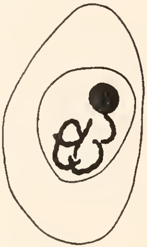
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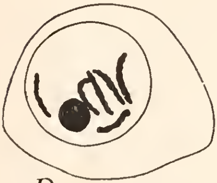
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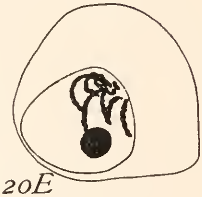
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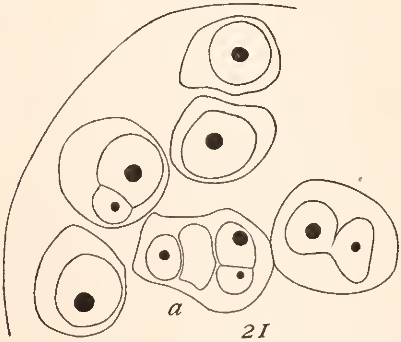

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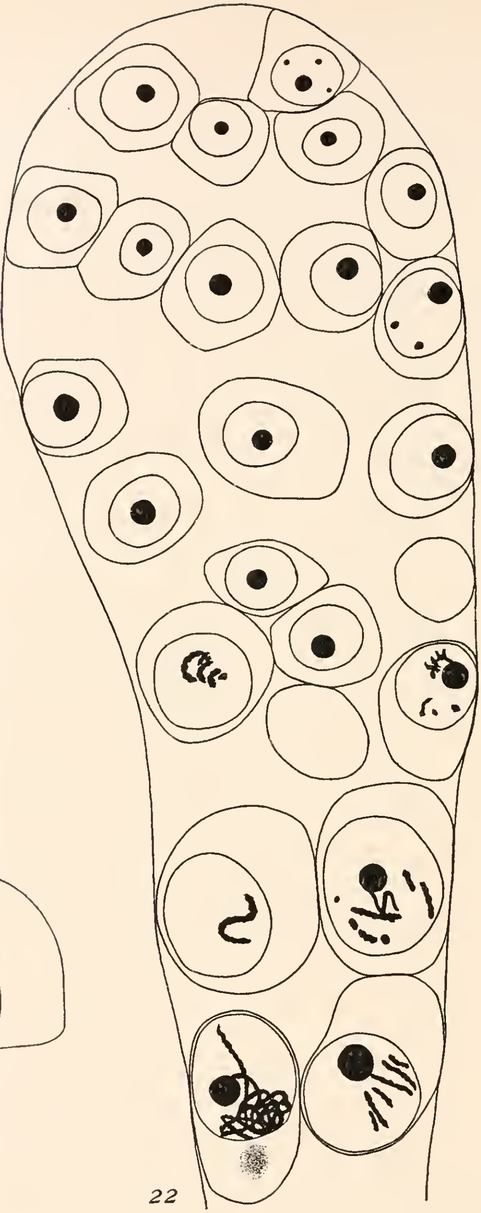
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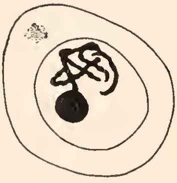
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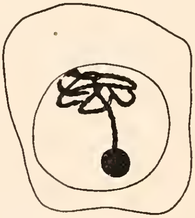

23B

Fig. 18. Fig. 19, *A*, 19, *B*. Figs. 19, *C*, 20, *A*. Fig. 20, *B*, 20, *C*.
Fig. 20, *D*, 20, *E*. Fig. 21. Fig. 22. Fig. 23, *A*, 23, *B*.

but in different proglottids. The chain in which mitosis was almost never seen in the ovarian development produced apparently as many eggs as others and these developed in the normal manner in the uterus.

VI. The Growth Period, "Synapsis" and Yolk Formation.

Although the later stages in the formation of the ova are not concerned directly with amitosis a brief description of these stages is added in order to demonstrate that nuclei which have passed through a long series of amitoses are quite capable of exhibiting the characteristic phenomena of typical germ cells. These stages in the development of the ova also appear to be identical in both species. All the figures except 22, 23 and 30, *A–D*, are from *M. expansa*.

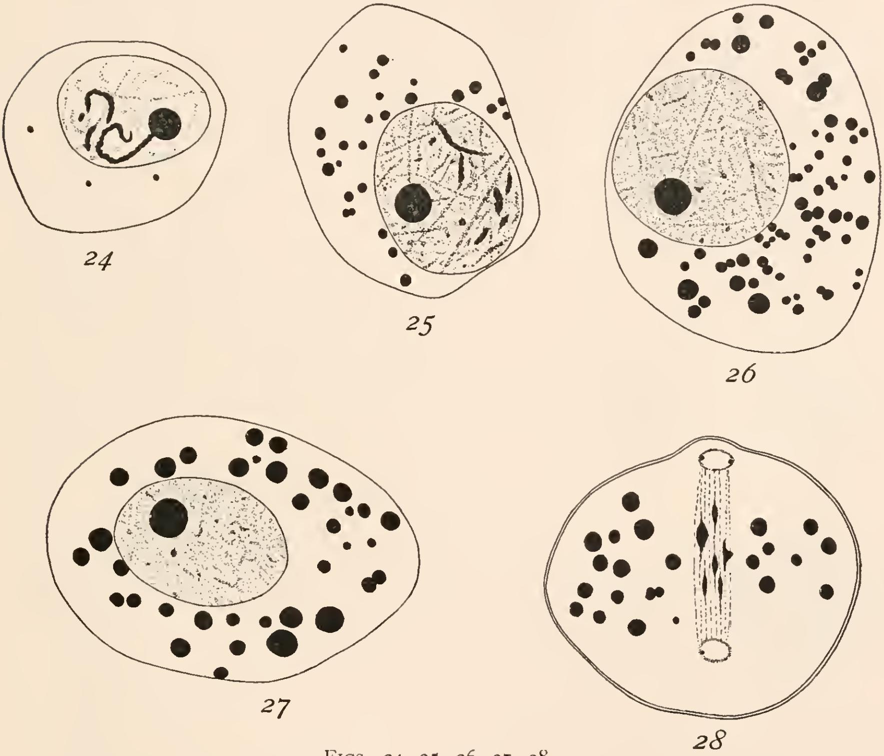
The oögonia at the end of the period of division are seen in Fig. 18. There is little difference in appearance between them and the dividing cells during ovarian development. The amount of cytoplasm is perhaps slightly greater, but this difference is not marked.

But now the nuclei undergo a sudden and remarkable change. The only deeply staining portions of the nucleus up to this time have been the nucleolus and frequently a few other granules (Fig. 18). Now the nucleus develops rapidly a large amount of chromatin. The earliest observed stages in this development are shown in Fig. 19, *A*–19, *C*. This change in the nuclear substance is accompanied by a great increase in size of both nucleus and cytoplasm. The chromatin soon shows itself in the form of a typical spireme (Figs. 20, *A*–20, *E*, 23, *A*, 23, *B*) which is commonly massed at one side of the nucleus (Figs. 20 *A*, 20, *C*, 20, *E*, 23, *A*, 23, *B*) and in most cases is visibly connected with the nucleolus. So far as can be determined the spireme does not appear to be formed from the substance of the nucleolus, since the latter increases in size like the other elements of the cell. This is clearly a typical case of what is commonly known as synapsis.

The appearance of these stages varies considerably with the degree of extraction of the stain. If extraction is carried farther than in Fig. 20 only small portions of the spireme or often only the karyosome retain the stain and the presence of the spireme would not be suspected. The writer is firmly convinced by long experience with this stain that it is of little or no value in determining differences of chemical constitution; size, density, and permeability seem to be the chief factors determining which parts shall retain the stain.

The spireme appears first in the central portions of the ovary adjoining the end of the oviduct and proceeds in all directions distally along the follicles. The follicle tips continue, however, to divide amitotically for some time after the other portions of the ovary have entered the spireme-stage. Fig. 21 shows the tip of a follicle at this stage. In the same ovary the cells near the oviduct were like those in Fig. 20. Fig 22 is a nearly longitudinal section through the terminal portion of a follicle showing the different stages at different levels. In all but one of the five cases with spiremes most of the chromatin is outside the plane of section. In this ovary all the cells have entered the spireme-stage except those in the terminal region of the follicles and division has already ceased there.

Together with the nuclear changes occur marked changes in the cytoplasm. The amount of cytoplasm increases in marked degree as the figures show. It also stains more deeply than before. During synapsis the nucleus is usually more or less asymmetrical in position. In many cases at least the greater amount of cytoplasm is on that side of the nucleus against which the spireme is massed. (Figs. 20, *A*, 20, *C*, 20, *D*, 20, *E*, 23, *A*, 23, *B*). It cannot be stated positively that this is always the case but it is certainly of frequent occurrence. After fixation in chromoxalic a regional differentiation of the cytoplasm is visible in many cases and is perhaps a characteristic feature. As noted above, the spireme usually becomes massed at one side of the nucleus: this is followed by the appearance in that part of the cytoplasm nearest the spireme of an area staining more deeply than any other part of the cytoplasm (Fig. 24, *A*). If extraction is carried too far this area is scarcely or not at all visible. It is comparable to certain of the differentiations which have been called yolk nuclei in other eggs and its appearance is followed almost immediately by the formation of yolk granules which in *Moniezia* are contained in the egg-cell itself. The first yolk granules to appear, however, are not confined to this region but are more or less scattered (Fig. 24). The natural conclusion from the sequence of events is that the change in nuclear condition is in some way correlated with the cytoplasmic changes and since the former precedes, that it is in some way responsible for the latter. Nuclear changes connected with yolk-formation have been described by many authors but it is necessary to review the various accounts. It seems probable these nuclear changes indicate an alteration in the metabolic processes and that they are concerned primarily with the increase of the cytoplasm and the deposition of yolk.



24 25 26 27 28

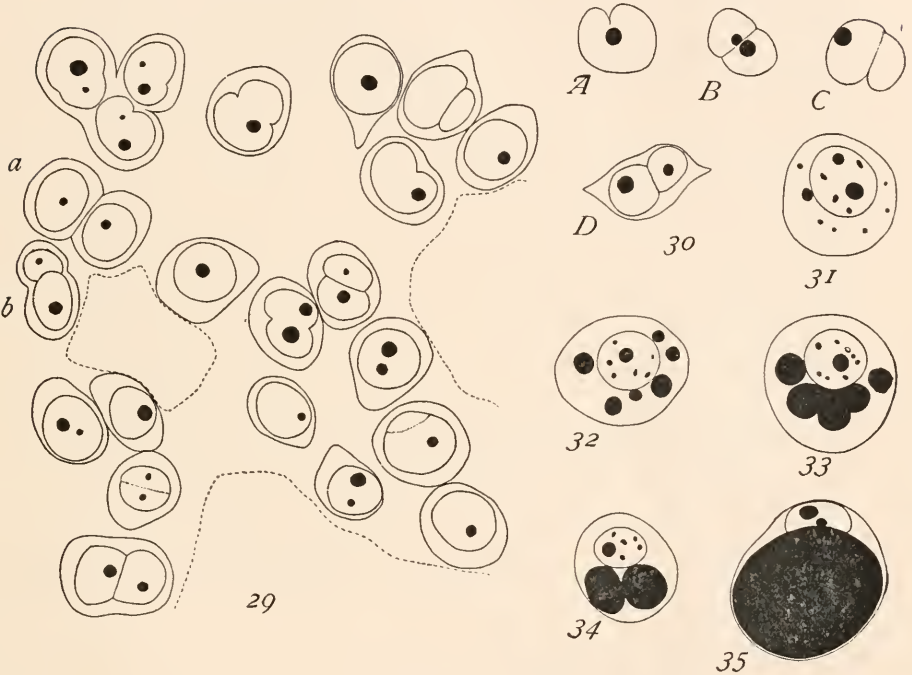
Figs. 24, 25, 26, 27, 28.

Figs. 24–27 show successive stages in yolk-formation. The granules formed first increase in size and others appear. Fusion of the smaller to form larger masses also occurs frequently. During yolk-formation the nucleolus remains apparently unchanged, but the spireme soon loses its deeply staining character and its substance appears to spread throughout the nucleus in a more or less recticular condition so that the nucleus resembles the nuclei of most eggs before maturation.

It is almost impossible to reproduce the nuclear structure of this period with any degree of exactness: moreover, it is not certain how far the visible structure is characteristic of the living cell and how far it is the product of fixation. An attempt has been made, however, in Figs. 24–27 to indicate the changes in nuclear structure. Although the spireme of earlier stages has disappeared the nucleus differs in appearance from the ovarian nuclei before the growth period in that a reticulum is now visible while in the earlier stages the nuclei were almost entirely homogenous in appearance except for the nucleolus and other granules. An account of maturation and fertilization will be given in another paper. At this time it need only be said that typical maturation spindles appear, but that it has been impossible to follow the process of chromosome-reduction. Fig. 28 shows the first polar spindle with its enormous centrosomes.

VII. The Development of the Vitellarium.

The "yolk-gland" differentiates from the posterior portion of the ovarian mass of proliferating cells. During the earlier stages its cells are indistinguishable from those of the ovary, but later they can be distinguished by their slightly smaller size. The nuclear division is almost wholly amitotic. Occasionally, however, though less frequently than in the ovary, a case of mitosis is seen. Division seems to be somewhat more rapid than in the ovary, amitoses being commonly more numerous in a given area. As in the ovary the nuclei of earlier stages are surrounded by very little cytoplasm and lie in the parenchymal tissue. Fig. 29 represents a group of nuclei at the stage when the yolk-gland is first distinguishable from the ovary. Numerous amitoses are visible. Fig. 30 shows several cases of division from a later stage. The process of yolk formation in these cells differs from that in the egg cells. It is not preceded by any marked increase in size of the nucleus or cytoplasm. No spireme has been observed but a number of deeply staining granules appear in the nucleus in addition to the karyosome. The yolk appears first in the form of small granules which increase in size and fuse, until the cell contains a single large spherical mass of yolk and the greatly reduced nucleus is flattened at one side. Successive stages of yolk-development are shown in Figs. 31–35. These cells apparently arise from the same primordium as the ova but have become specialized in the direction of yolk-production. The process of yolk-formation is not identical with that in the egg but this difference is probably correlated with the extreme specialization.



Figs. 29–35.

VIII. Conclusion.

Extended discussion is better postponed until other data have been presented, but the most important facts of this paper may be briefly stated as follows.

Nuclear division in the development of the female reproductive organs of *Moniezia expansa* and *M. planissima* is predominantly amitotic though typical mitoses occur, their frequency varying apparently in different chains and in different proglottids.

After the long period of repeated amitotic division the nuclei pass through the characteristic mitotic maturation divisions and the cells form typical ova.

The process of amitosis consists in the formation of a constriction in some part of the nucleus or of a "nuclear plate" or membrane across some portion of the nucleus and the separation of the nuclei thus marked off and later of the cytoplasm about each. Each part thus separated usually possesses a visible nucleolus at the time of formation of the membrane.

The fact that the female germ-cells may arise by a long series of divisions almost wholly amitotic is of considerable theoretical importance. There is no room for doubt that the fate of a cell may be the same, whether it divides mitotically or amitotically during developmental stages. It is also very difficult to understand how anything like individuality of the chromosomes can be maintained in this case during the development of the germ-cell.

The two forms of division may occur side by side in the same tissue and at the same stage, but their relative frequency may vary in different individuals and in different proglottids. It seems probable, therefore, and other data will confirm this conclusion, that the form of division is determined by the conditions to which the cell is subjected.

Hull Zoölogical Laboratory,
University of Chicago,
August, 1906.

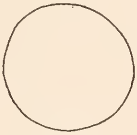
PROBABLE DIMORPHISM OF THE EGGS OF AN ARANEAD.[1]

THOS. H. MONTGOMERY, Jr.

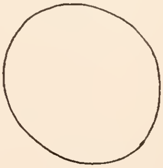
On comparing the eggs from cocoons raised in captivity of the common spider, *Theridium tepidariorum*, C. K.,[2] I was struck by the fact that eggs of the same age but from different cocoons may be of distinctly different volumes. That is to say, all the eggs of one cocoon may be larger or smaller than all the eggs of another cocoon made by the same spider.

In this comparison only such egg batches were considered, of which I had records as to the exact hour of oviposition. Further, comparisons were made only of eggs preserved by the same fixative, hardened in the same way, and preserved in the same grade of alcohol; in all cases the cocoons were opened and the eggs dropped into the fixative. Then all the spiders came from one locality, and all lived under the same conditions of captivity in the month of August, 1906.

The following cases exhibited different volumes of the eggs of successive cocoons made by particular spider individuals:



819.



753.

1. One spider formed three successive cocoons, nos. 752 (9th August), 818 (14th August), and 872 (19th August). Every egg of no. 752 (fixed at the age of twelve hours) was markedly larger than any egg of no. 818 (fixed at the age of fifteen hours) and no. 872 (fixed at the age of nineteen hours). The difference in these egg sizes is shown in the accompanying figures.

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, no. 81.

[2] *Vide* my preceding paper in this journal, "The Oviposition, Cocooning and Hatching of an Aranead, Theridium tepidariorum, C. Koch."

2. Another spider made two cocoons, nos. 725 (8th August) and 821 (15th August). All the eggs of no. 725 (fixed at the age of two hours and twenty minutes) were markedly larger than any of the eggs of no. 821 (fixed at the moment of oviposition).

3. Another spider made cocoons nos. 770 (11th August) and 905 (18th August); the eggs of no. 770 (fixed at the age of 50 minutes) were all markedly smaller than the eggs of no. 904 (fixed at the age of 60¼ hours).

4. A fourth spider furnished cocoons no. 726 (8th August) and no. 850 (17th August). The eggs of no. 726 (fixed at the age of 3½ hours) were all markedly smaller than those of no. 850 (fixed at the age of 3 hours).

The preceding series of cases show that successive cocoons may have eggs of the same size; or, and this is what more particularly interests us, that all the eggs of one may be larger or smaller than all the eggs of another. It will be also noticed (cases 1 and 4) that eggs of younger age may be larger than eggs of maturer age. Indeed, there is probably no change in the volume of a given egg from the time of oviposition up at least to the time of appearance of the limbs; accordingly, individual growth of an egg is not a factor entering in to disturb our conclusions as to these voluminal differences, since we are considering only stages antecedent to the appearance of limbs.[1]

To estimate the comparative egg volumina I placed the eggs side by side under the lens, and judged their difference ocularly, always comparing the smallest of a larger batch with the largest of a smaller batch. It would not be possible to estimate relative volumes by determining the number required to fill a given space, without first dissecting off the envelopes of each egg.

Now the variability of volume is usually of small amount in any given cocoon, that is, all the eggs of a cocoon are large or all small in most instances. But there are frequent exceptions to this. Thus one female produced three cocoons: nos. 722 (8th August), 800 (12th August) and 856 (17th August), all the eggs of no. 856 (fixed at the age of 26 hours) and almost all the eggs of no. 800 (fixed at the same age) were larger than the eggs of no. 722 (fixed at the age of 1¾ hours), but a few eggs of no. 800 were as small as the eggs of no. 722. The same held for the successive cocoons of four other spiders. Then in one cocoon collected in the wild state, not raised in captivity, there were 596 eggs (the largest number I have found in any single cocoon); most of these could, with certainty, be ranked as large eggs (about 478) while about 118 of the eggs were clearly small eggs, but a few were intermediate in size between these two groups. The latter case is important in showing that while intermediate sizes may occur between the large and the small eggs, in the same cocoon or in successive cocoons, the intermediates are very few in number compared with the extremes, a condition that would not occur in simple individual variation.

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[1] During oviposition the eggs are polygonal, but usually within a few minutes all become rounded (slightly ovoidal), as has been noted by Balbiani: Mémoires sur le développement des Aranéides, 1873. *Bibl. de l'École des Hautes Études*, T. 7.

The conclusions permitted by these observations are as follows: This species of *Theridium* produces large eggs and small eggs; in one cocoon all may be large or all may be small, or in any one cocoon both kinds may occur; intermediates in size are relatively very infrequent. Such a difference of volume might be termed "dimegaly" for convenience, especially when no structural differences are found or known to accompany this difference in volume. But there is a possibility if not a probability that this dimegaly may be dimorphism, and that females develop from the large eggs and males from the small ones. The adult female spiders are considerably larger than the adult males, notably with regard to the dimensions of the abdomen. The occurrence of occasional intermediates between the two kinds of eggs may be readily explained by the fact that in each kind of eggs there is always some individual variation in volume, in conjunction with the assumption that the smallest extremes of the larger kind may not be larger than the largest extremes of the smaller. In those cases where some cocoons contain only large eggs, others only small ones, there would then be instances where some cocoons produce only females and others only males. Further, if this dimegaly is really dimorphism, a conclusion, that we are tentatively maintaining, then in a succession of cocoons made by the same spider there would be batches of female eggs only alternating with batches of male eggs only; I did not have a sufficient number of cocoons from any one female to determine what is the regularity of this succession. Or again both kinds of eggs may occur in the same cocoon, and perhaps future observations will show that the first cocoons contain only large (or small) eggs, the next succeeding eggs of both kinds, and the last cocoons only small (or large) eggs.

Whether this dimegaly is true sexual dimorphism can be decided only by examining the genital organs of the hatching spiderlings since there are no external sexual differences apparent in the young, which would require much labor; or by raising all the spiderlings to maturity, a method that would require still more time and patience. But one of these methods must be tried in order to finally demonstrate whether this is true sexual dimorphism of the eggs.

If the small and large eggs of *Theridium* are really male and female eggs, and it must be admitted that there is a probability of this, then here is another instance of two kinds of eggs to be added to those already known, namely, the cases of the Aphids, Rotatoria and *Dinophilus apatris*. Adult sexual differences in size are very marked in many spiders, the male is probably always somewhat the smaller, and in many species, particularly among the Argiopidæ, Theridiidæ and Thomisidæ the disparity in size of the sexes is most striking. It would be of interest to examine this point in the case of the common orb-weaver *Argiope cophinaria* (Walck.), where the male may be less than one fiftieth the volume of the grown female, here if any where there should be marked dimorphism of the eggs; and in species of the genus *Acrosoma*. The common *Epeira labyrinthea* Hentz would be especially favorable because it places its cocoons in a string in the order of their making.

THE "ACCESSORY CHROMOSOME" OF ANASA TRISTIS.

KATHARINE FOOT AND E. C. STROBELL.

In the *Quart. Journ. Mic. Sci.*, Vol. 48, 1905, Professor J. E. S. Moore and L. E. Robinson writing on the spermatogonesis of *Periplanata Americana* claim that the nucleolus of the first spermatocyte is undoubtedly the homologue of the structure described by Paulmier ('99), Montgomery ('01), and McClung ('02), in different forms as one or two of the spermatogonial chromosomes. It's morphological resemblance to a chromosome shown by its frequent elongate form, Moore and Robinson attribute to mechanical influences and claim that normally it is spherical like the nucleolus. In three recent papers Professor E. B. Wilson ('05, '06) has given special attention to this structure in a number of forms and the above interpretation of Moore and Robinson he ascribes to superficial work.

A study of the spermatogenesis of *Anasa tristis*[1] has convinced us that for this form the interpretation of Moore and Robinson is correct, that the nucleolar-like structure of the rest stage is the homologue of the nucleolus of the egg, that it is not a chromosome, as claimed by the three cytologists who have investigated this form.

In 1899 Paulmier identified this body of the rest stage with the two small spermatogonial chromosomes which Wilson has aptly named the "microchromosomes."

Montgomery in 1901 supported Paulmier in this interpretation, but in 1906 he changed his position and now supports Wilson in identifying this structure — the so-called "chromosome nucleolus" — as one of the larger spermatogonial chromosomes an unpaired spermatogonial chromosome, called by Wilson the odd or "heterotropic" chromosome. Both these investigators claim that it divides only in the first division, in the second division, passing undivided to one pole of the spindle.

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[1] We are indebted to the courtesy of Dr. P. R. Uhler for identifying our material.

In Wilson's "Studies on Chromosomes, Nos. II. and III.," he has published several text figures of *Anasa tristis*, including the stages from the "contraction phase" of the first spermatocyte to the anaphase of the second spindle, and also four spermatogonial groups in which he figures an odd number of spermatogonial chromosomes, *i. e.*, 10 pairs, and one large unpaired univalent, the odd or "heterotropic" chromosome.

A study of a large number of smear preparations of the testes of *Anasa tristis* has forced us to the conclusion that in the spermatogenesis of this form there is no so-called "accessory chromosome" — no odd, "heterotropic" chromosome — that the so-called "chromosome nucleolus" of the rest stage is the homologue of the nucleolus of the egg, that in its form and time of disappearance it bears a striking resemblence to the plasmosome of the egg of *Allolobophora fœtida*. Our observations and interpretations are so at variance with the conclusions reached by the three cytologists who have studied this form, that we would hesitate to take issue with such competent authority were we not able to support our observations by a very large number of photomicrographs of the preparations. We have already nearly 200 photographs which seem to demonstrate beyond question the following points.

That the so-called "chromosome nucleolus" of the resting spermatocyte is morphologically the equivalent of a nucleolus, that it is not a chromosome. Wilson has emphasized the evidence of morphological likeness in his Fig. 2, *b* and *c* ("Studies on Chromosomes, II."), in which he shows a structure which he interprets as a chromosome and which has a marked morphological resemblance to his sketch of the "chromosome nucleolus" in his Fig. *a*.

It is significant that in Wilson's three figures of this early prophase only 6 of the 11 bivalents are shown in two of his drawings and only 7 in the third. In fact, not one of the investigators of this form has given a single figure of this stage in which *all* the eleven chromosomes are shown.

In our photographs, on the contrary, *all* the 11 bivalents are in evidence, and not one of them resembles in the least the "heterotropic" chromosome figured by Wilson. This holds true for hundreds of cells in which all the eleven bivalents are present and clearly defined.

It is only when the chromosomes or parts of chromosomes are abnormal that they show a condensed chromatin mass, or masses, suggesting a resemblance to a nucleolus. In many cases one of the arms of a cross-shaped chromosome will resemble a round dense nucleolus and this may appear in from one to five of the crosses, and again both arms, or the entire cross may have degenerated into a compact, deeply staining mass of chromatin. We have a number of photographs of connecting stages between these extremes, and they leave no doubt that the normal chromosome resembles in no way a nucleolus.

Our preparations also demonstrate that the "chromosome nucleolus" like the plasmosome of the egg of *Allolobophora fœtida* has disappeared, as a rule, when the chromosomes are formed (early prophase) very rarely persisting until after the chromosomes have attained their definite shape.

Our smear preparations further demonstrate the absence in the resting spermatocyte of any other structure which can be interpreted as a nucleolus. We approached the study of this form with the hope of being able to identify a structure in the male cell which could be interpreted as the homologue of the "accessory nucleolus" of the egg,[1] but we have found no structure sufficiently pronounced or constant to justify our interpreting it as an "accessory nucleolus."

Paulmier ('99), Montgomery ('01) and Wilson ('05–'06) have all indicated a second nucleolar-like structure in the resting spermatocyte which they interpret as the true plasmosome, but we have been unable to demonstrate a second nuleolar-like structure in our smear preparations. However in sections of testes fixed with Hermann's fluid and stained with iron hæmatoxylin and with anilin stains, we often find nuclei of resting spermatocytes in which a second nucleolar-like structure is differentiated, but the complete absence of such a feature in our smear preparations, makes us hesitate to interpret these two structures as the homologues of the plasmosome and accessory nucleolus of the egg.

Again our preparations demonstrate that the so-called univalent "heterotropic" chromosome is distinctly a bivalent. Its constant bivalent character indicates that it represents in value two spermatogonial chromosomes and not one, and when this chromosome is first formed its bivalent character is much more pronounced than at the later prophase stages. Our photographs however support Wilson in his claim that it appears only *exceptionally* as a tetrad — as a rule this and the micro-chromosomes appear bivalent, while all the others show a marked tetrad character. The frequent eccentric position of this bivalent chromosome, outside the characteristic ring arrangement of the chromosomes in the late prophase, seems to warrant suggesting "eccentric" chromosome as a convenient descriptive name for this special chromosome.

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[1] Foot & Strobell, 1905.

Individuality of the Chromosomes. — Our preparations show a marked individuality of the chromosomes, and in this support the observations of Paulmier, Montgomery and Wilson. Several of the chromosomes can often be clearly identified during the prophases, metaphase and anaphase, though a comparison of a large number of photographs demonstrates that the *form* is not constant. For example, at a definite prophase, 7, 8 or 9 of the 11 bivalents may be clear and sharply defined crosses, while again in the same stage we may have all rods or only 1, 2 or 3 crosses, this indicating that the cross type is not invariably associated with any one chromosome.

During the growth period the chromosomes certainly lose their individuality as completely as in the case of *Allolobophora fœtida*, and we have therefore no positive proof that each bivalent of the prophase represents the same chromatin that formed a pair of the spermatogonial univalents. There is a certain degree of constancy in the relative sizes of the chromosomes, although a definite chromosome may differ greatly in size in different cells at the same stage of development. This may be due in some degree to the technique, but this difference is often so great that we feel convinced it is probably due at least in part, to an actual difference in the size of the individual chromosomes.

Montgomery ('06) observed an inequality in the size of the two microchromosomes, but in our preparations we do not find any support for this observation.

Plane of the First Division. — In many cells in which all the chromosomes are clearly defined a transverse division for *each* chromosome can be plainly demonstrated by one photograph. In many other cells, however, it can be as clearly determined that the "eccentric" chromosome divides longitudinally while all the others divide transversely. It may be stated, as a rule, that the "eccentric" chromosome divides longitudinally, though many exceptions can be demonstrated.

The Lagging Chromosome of the First Division. — At the late anaphase or early telophase of many of the first divisions, it can be demonstrated that one of the chromosomes has divided at a later period than the others. It may be seen between the two poles in all stages of separation — sometimes the entire bivalent will be between the poles, its two univalent halves having just separated or one univalent may have reached one pole while the other half still lies midway between the poles. We have several photographs in which a lagging chromosome is shown while all the other chromosomes can be counted, thus an error in interpretation is quite impossible. As a rule this lagging chromosome appears to be the "eccentric" chromosome, though it cannot be demonstrated that it is invariably the same chromosome which lags in division. We have not found this phenomenon with sufficient frequency to justify our interpreting it as a constant feature of this division. We think the condition exceptional, though not necessarily pathological.

The Lagging Chromosome of the Second Division. — The second division shows a phenomenon which appears to us to be the equivalent of the one just described for the first division. It is more frequently found for the second spindle than for the first and much more difficult to interpret, as these spindles are so exceedingly small and the chromosomes so closely crowded together, that cases are rarely found in which *all* the chromosomes are in evidence and the true value of the lagging chromosome can be safely interpreted. We have a few photographs which we think throw some light upon this point. *All* the chromosomes at each pole are demonstrated and the lagging chromosome lying midway between the poles, in several cases shows a distinct transverse constriction. In one preparation the two halves have separated, and in another the two halves have reached opposite poles of the spindle, though in these cases the chromosomes at the poles are too crowded to be counted. We interpret this lagging chromosome as a univalent, being equal in value to all the other chromosomes of the second spindle, just as in the first spindle we interpret the lagging chromosome as a bivalent — both cases indicating simply a retarded division of one of the chromosomes.

Our preparations do not support Wilson and Montgomery in their observation that the lagging chromosome goes over undivided to one pole of the second spindle, and we are therefore unable to follow them in supporting McClung's theory of the dimorphism of the spermatozoa.

If these authors are correct in interpreting this lagging chromosome as only half of a univalent, what is the significance of the frequent transverse constriction? What can such a constriction mean but foreshadowing a division? and this interpretation is supported by the cases in which the division of this chromosome is actually demonstrated. We do not interpret the presence of a lagging chromosome in the first or second spindle as necessarily an abnormal condition though it may be a step in that direction, for we have seen unmistakably pathological spindles where sometimes one and sometimes two chromosomes pass to one pole undivided. We have photographs of some of these spindles and their pathological character can be readily recognized. We also have examples of such unequal, abnormal separation of the chromosomes in the first and second spindles of *Allolobophora fœtida*.

Spermatogonial Chromosomes. — Paulmier ('99) who was the first to study the spermatogenesis of this form interpreted the number of spermatogonial chromosomes to be 22 and in his Fig. 9 has reproduced one of his sections in which 22 chromosomes are clearly represented.

Montgomery ('01) supports Paulmier in his estimate of the number of spermatogonial chromosomes and in his Fig. 74 gives a very clear demonstration of this number.

Wilson in his "Studies on Chromosomes, No. 1," corrects this original count of the spermatogonial chromosomes with such positive assurance[1] that we have great hesitation in questioning his results and would not presume to do so were it not possible to corroborate our observations with photomicrographs in which 22 chromosomes can be counted without any question. We realize in common with all cytologists the difficulty of getting a correct count of so large a number of small bodies crowded into a contracted space. If 2 or more chromosomes are in such close contact that their line of separation is obscured a correct count is impossible. It is certainly possible to find cells in which only 21 chromosomes can be differentiated and still easier to find cells in which only 20 or 19 are defined. It is much more difficult to find each chromosome so distinctly isolated that all can be demonstrated in one photograph.

Wilson ('05–'06) corroborates his original count of 21 spermatogonial chromosomes and illustrates this point in four sketches. Montgomery ('06) in his last paper withdraws his earlier estimate of the number and supports Wilson's results, figuring 21 chromosomes in his sketch No. 151.

In view of this weight of authority we do not feel inclined to be in the least dogmatic in our estimate of the count of the chromosomes, but our preparations certainly justify us in maintaining that it is possible to demonstrate 22 spermatogonial chromosomes in *Anasa tristis*, as we shall show later in photomicrographs of the preparations themselves.

December 1, 1906.

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[1] "Since this paper was sent to press I have determined beyond the possibility of doubt, I think, that the number of spermatogonial chromosomes in *Anasa tristis* is 21 not 22 as given by both Paulmier and Montgomery. This result is based on a study of a large number of preparations and careful camera drawings of more than 20 perfectly clear metaphase figures have been made. All without exception show 21 chromosomes, and I have sought in vain for even a single cell that shows 22."

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BIOLOGICAL BULLETIN

THE HABITS AND MOVEMENTS OF THE RAZOR-SHELL CLAM, ENSIS DIRECTUS, CON.

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Many of the older naturalists have called attention to the sensitiveness and remarkable activity of this form, and the consequent difficulty that is sometimes experienced in capturing it. Some of the observations are not strictly accurate but they have served to call attention to its adaptation for a burrowing life. Even the uncommon shape of the animal indicates this adaptation

The species under consideration is to be found more or less abundantly all along the eastern coast of the United States. It is best known on sandy flats from which most of the water flows at low tide, where specimens may be dug with spade or clam-hoe. In some localities, as in restricted areas around Woods Holl and North Falmouth, Massachusetts, where most of these observations were made, the animals are quite abundant, and in such places one may find the protruding posterior ends of the shells, or see the siphon openings of the undisturbed individuals. They readily take alarm and even a slight jarring of the mud of the bottom in their vicinity serves as a signal for them to instantly disappear. It is this sudden disappearance that has attracted wide attention, and has given the impression that the animals are exceptionally hard to capture (5 and 6).

The species is probably not restricted to very shallow water. Specimens are not often taken in a dredge, but the position that they occupy buried in the rather hard sand or mud of the bottom, makes their capture unlikely. Young specimens, from a millimeter to a centimeter in length have been taken in large numbers near South Hapswell, Maine, in from ten to thitry feet of water, by means of a fine wire dredge. Supposedly where conditions are right for individuals to grow to a centimeter in length, larger ones would thrive also.

The usual position for undisturbed specimens is with the posterior ends of the shells protruding just above the surface of the mud. Sometimes specimens are found with several centimeters of their shells protruding, but this is not very common. Again all that indicates the presence of a specimen, may be the depression at the spot where the animal has disappeared. The most conspicuous parts of a specimen in its normal position are the siphons, the openings of which appear as nearly round apertures surrounded by tentacles (Fig. 5). Close observation is necessary to see such specimens, as the color of their siphons is almost exactly the same as the bottom where they occur.

Specimens that have their posterior ends protruding above the surface of the mud seem to be more common where the water has drained entirely away than where the flats are still covered with water. Shells are not uncommon on the flats and shores, but they are seldom found embedded in the position that the animals occupy during life. These observations are of especial interest in view of the fact that animals in aquaria quite universally push up out of the mud before they die. The heat of the sun on the bare mud flats is probably disturbing and may cause them to react in the same way they do before they die. If animals react in nature as they do in aquaria, it is natural that the shells of dead animals should be found on the surface.

Specimens are not easily studied in their native places because of ripples on the water and because the character of the bottom makes it hard to approach closely without disturbing them. Walking on the mud near them will cause them to withdraw their siphons or disappear beneath the surface. It has accordingly been more satisfactory to study specimens in aquaria that contain several inches of sand or mud from the bottom from which the specimens were obtained. The animals do not live well in aquaria, even when supplied with running water, but for several hours after they are collected, they remain very active and seem to be quite normal.

If a specimen is touched it will either withdraw its siphons anp remain quiet until disturbed again, or it will immediately vanish beneath the surface of the mud. If a specimen is grasped and pulled upward there is an immediate response that is so powerful that the animal frequently escapes and disappears. This fact Tryon (6) mentions, saying: "It may often be seen at low tide projecting a little above the level of the sand but, if touched or disturbed, it descends with astonishing rapidity and force, much to the amazement of him who may lay hold of it thinking to make an easy capture." These observations indicate that the animal probably habitually keeps its foot protruded some distance out of the shell to be ready for disturbances.

Specimens taken in firm sandy soil, where the depth can be noted, are frequently found several inches below the surface. Verrill and Smith (7) report that this species digs somewhat permanent burrows that extend nearly perpendicularly into the sand to the depth of three feet, and Woodward (8) states that the animals never voluntarily leave their burrows. I have never been able to demonstrate permanent burrows in the localities where I have worked, but the usual muddy character of the bottom was not satisfactory for the purpose. I doubt, however, if such burrows are habitually constructed. The character of the bottom where they live is frequently not suitable for permanent burrows unless something like a tough secretion is added to the mud to keep it in place, and such a secretion does not seem to be formed. Specimens in aquaria never seem to construct anything like permanent burrows.

As individuals are known to burrow to some depth it is probable that in digging for them, those in the immediate vicinity are disturbed and burrow beneath the reach of the shovel. Verrill and Smith (7) call attention to how easily disturbed they are and say: "When thus alarmed it is generally useless to try to dig them out, for they quickly descend beyond the reach of the spade." Dr. J. Gwynn Jeffreys as quoted by Tryon (6) reports them as having exceptional powers for detecting disturbances. He says: "They are evidently sensible to vibratory movements in the air, as well as on ground, taking alarm at greater or less distances according to the state of the atmosphere and the direction of the wind." It is hard to verify these observations and I am inclined to think they are not accurate, but there can be no doubt that specimens are easily disturbed by vibrations of the bottom. That they habitually burrow to some depth is indicated by the fact that after the first half dozen trials, specimens are not usually obtained without going to another spot several feet away, although the first trials may have resulted in one or more specimens each.

When dug from the mud, individuals frequently leap or swim. In leaping the shell may be thrown several inches by the action of the foot. In swimming the animal progresses posterior end first and large specimens may swim several feet before stopping. The foot is always active while the animal is swimming. These activities, together with the movements of burrowing, will receive attention later.

Before describing the movements it is desirable to call attention to some points of anatomy.

The shell is of nearly even diameter both dorso-ventrally and laterally (Figs. 1 and 4), throughout its length, except very near its ends. Anteriorly it contracts in both directions a little, but the anterior margins of the shell valves remain wide apart even when they are in contact along their ventral borders (Fig. 6). This leaves ample space for the protrusion or withdrawal of the foot when the shell is closed. The anterior margins of the lobes of the mantle are thickened and extended, so, when the foot is withdrawn into the shell, these flaps cover the opening between the shell valves (Fig. 6). When the foot is extended the flaps are spread apart and form a collar around the foot, the free margins of which are in contact with the foot (Figs. 1 and 4). The collar is thick and muscular, being well supplied with the radial pallial muscles, and as it is held tightly against the foot, forms a very effective scraper, that cleans the foot so mud is not drawn into the shell with it. The cilia covering the foot no doubt aid in loosing the mud so it is easily scraped off. The flexible collar adapts itself to the shape of the foot so its margin is applied to the surface of the foot until its very extremity is drawn into the shell (Figs. 6 and 7).

The posterior end of the shell narrows laterally, but here again the margins of the shell valves are wide apart when the ventral edges of the valves are in contact (Fig. 5). This makes it possible for the siphons to be at least partially extended when the shell is closed. The reason for this arrangement is found by studying the movements of burrowing and swimming.

The siphons have nearly circular or slightly elliptical openings, and are separated near their extremities. The whole posterior end of the mantle, bearing the siphons, may be protruded some distance beyond the posterior end of the shell. Sense tentacles surround the siphons near their bases and occur on the mantle dorsally and ventrally near the posterior end, where the mantle is exposed between the shell valves (Figs. 2 and 5). Small sense tentacles occur on the surfaces of both siphons, and the branchial siphon bears a number along its extremity that tend to radiate in over the opening of this siphon. The margin of the cloacal siphon has no tentacles. Verrill and Smith have (7) described a definite arrangement for the tentacles but it is doubtful if this arrangement always holds.

The ventral margins of the mantle lobes are united throughout their length except near the middle of the length of the animal, where a small opening remains that is situated just posterior to the retracted foot (Fig. 4). This opening is surrounded by a single row of sense tentacles. Except for this opening, the opening through which the foot is protruded, and the openings of the siphons, the mantle forms a closed chamber.

The united mantle margins are very muscular, being provided with strong circular and radial pallial muscles that are very similar to the muscles of the mantle margins in *Solenomya* (2) where they serve very much the same purpose, that is, to close the shell tightly and to obliterate a portion of the mantle chamber. Like *Solenomya* the valves of the shell are covered with a very heavy, elastic cuticle that is extended beyond the calcareous margins. Mud does not readily adhere to this cuticle. When the valves are closed the cuticle is bent in over the hard margins of the shell (Fig. 8), thus allowing the united margins of the mantle to be withdrawn. Probably this elastic cuticle aids in opening the mantle chamber when the muscles relax, as is undoubtedly the case with *Solenomya* (2), but the effect in this form is certainly much less than in *Solenomya*.

Both adductor muscles are present. The anterior adductor is very large and strong. The posterior adductor is quite small and does not seem to function actively. The united margins of the mantle posterior to the ventral opening are especially muscular and seem to replace the posterior adductor in function to a marked extent.

The foot, when retracted into the shell, is nearly cylindrical and together with other organs completely fills the part anterior to the ventral opening in the mantle. The dorsal portion of its extremity is pointed, and a slight ridge marks the boundary of what may be called the sole (Fig. 2). The foot is very powerful and remarkably active, its movements being very unlike the slow movements of the foot in most lamellibranchs. It may be thrust from the anterior end of the shell to a distance exceeding one half the length of the shell, and in this position the end may be swelled into a knob or bulb that considerably exceeds the diameter of the shell (Fig. 1). The knob is not cylindrical but is extended dorso-ventrally and laterally and the free extremity or sole is comparatively flattened. In this swollen condition the end of the foot forms a very efficient anchor, as will be found by grasping a shell and trying to withdraw it from the mud. The resistance of the expanded foot is so great that the foot is frequently torn away from the shell when the shell is jerked quickly (7). The foot is attached to the shell by two pairs of foot muscles, both of which are strong and aid in withdrawing the foot into the shell. With the end of the foot anchored, the obvious result of the contraction of these muscles is to pull the shell into the mud up to the position of the bulbous portion of the foot.

For our present purpose it is not necessary to give more attention to the anatomy of the animal, and we will proceed at once to the study of the movements.

Burrowing. — The movements of burrowing may be best studied either in specimens placed in shallow dishes of sea water, which are very likely to execute the movements soon after they are placed in the water, or in specimens held with the anterior end pointing downward and stimulated to activity by stroking the sense tentacles around the ventral opening in the mantle and around the siphons. Specimens in the water are more normal in their movements than the specimens held and stimulated as described. Apparently the action of gravity may cause the held specimen to protrude its foot and to partially expand the end of it, in which position the foot may remain quiet for some time. When the movements are active they are essentially the same in both cases. The foot is slowly protruded with the pointed tip working as if trying to bore into the mud, ending each time with a dorsal thrust. These movements are continued until the foot is fully extended. During this extension the end of the foot is kept small, the point is directed well forward, and the general diameter of the protruded part of the foot is decidedly less than its normal diameter when at rest. When the foot reaches its greatest extension, the end is suddenly swelled into a great bulb, more than twice the diameter of the remainder of the foot (Figs. 1 and 4) and the whole foot becomes very rigid. That this result is attained by injecting blood into the foot may be readily proved by sticking spring forceps into the end of the foot so the spring will hold the wound open, and stimulating the foot to activity by stroking the tentacles as before described. When the foot starts to become active the wound begins to bleed rapidly, and when the final effort to swell the end of the foot is made, the blood rushes out in a great jet, but the swelling is slight. A simple incision does not answer as well, as the contracting muscles seem to close the wound more or less perfectly.

The instant that the swelling of the end of the foot is complete, a process that takes place so rapidly as to be almost startling, the retractor muscles pull the foot back to the shell with a jerk, the end remaining swollen until it reaches the shell (Fig. 7). It is then reduced in size and either withdrawn into the shell or extended in the beginning of a new burrowing movement.

While the foot is being extended the shell valves are allowed to gap apart and the siphons and ventral opening in the mantle are kept more or less widely open. Just before the final sudden retraction, the siphons and ventral opening are all tightly closed, and kept so until retraction is complete. The result is that the water in the mantle chamber is discharged through the opening through which the foot is extended, between the collar and the foot. Whether the water escapes all around the foot or only ventral to it, where the contact of the collar is poorest, has not been determined, but the jet of water is quite powerful. When the shell is embedded in the mud, each retraction of the foot, squirts the water against the mud ahead of the shell, the shell is decreased in diameter by being closed, and the mud is dislodged and washed up the sides of the shell where it may be seen raising after each downward movement of the shell. The action is similar to the pile driver that opens a way for the pile by a somewhat similar stream of water.

The burrowing movements may follow each other quite rapidly but the extension of the foot is never very rapid, as it must be carefully worked into the mud to keep from forcing the shell back. The opening of the shell just before the extension of the foot, tends to embed it more firmly and thus to hold it in position while the foot is being worked into the mud.

A specimen laid on its side on mud, has no difficulty in gaining a hold with its foot that enables it to right itself and start the anterior end into the mud. Burrowing is then normal, and the shell is soon completely buried. The time necessary for a specimen to completely bury itself varies with the character of the mud. In soft mud the thrusts may be rapid and few are needed, in hard sand the thrusts will necessarily be slower and more movements are required, but even in such material the animal will disappear very promptly. When the animal is laid on its side on such sandy mud as that in which it usually lives, one movement will frequently suffice for it to right itself, and four or five more will carry it out of sight. The time necessary for this may be less than half a minute. Embedded as the animal usually lives, a single retraction takes it out of sight and away from enemies.

Swimming. — It is more difficult to study the movements of swimming, as animals swim only occasionally, and then generally immediately after being dug, and the movements of parts of the animal, and the animal as a whole, are so rapid as to make accurate observations difficult. The following points have been determined however and, from these, conclusions may be drawn: (1) The animal progresses posterior end foremost; (2) movements are by jerks, each jerk carrying the animal one or more times its length; (3) the foot is very active, being thrust out and withdrawn repeatedly. The outward thrust is comparatively slow but the withdrawal is extremely rapid; (4) apparently the valves of the shell are drawn together every time the foot is retracted; (5) each movement of the animal as a whole, corresponds to the period of retraction of the foot.

In describing the movements of burrowing it has already been mentioned that water is thrown from the shell, through the opening through which the foot is protruded, every time the foot is retracted into the shell. Each jet is caused by closing the other openings into the mantle chamber and driving the water out by pulling the foot in, by closing the shell by the contraction of the adductor muscles and the united margins of the lobes of the mantle, and by drawing the mantle margins, with the shell cuticle to which they are attached, into the mantle chamber (Fig. 8). The resultant action is to drive out most of the water that was between the valves of the shell, as nearly all of the space is now occupied by organs of the body. As all of the openings except the one around the foot are held closed, a very strong jet of water must be forced out around the sides of the foot. This is sufficient to cause the movement of the animal in the opposite direction. Many muscles, all of which are powerful, are used in this action, and as the water is thrown through a small opening between the muscular collar and the foot, the resulting force is considerable. The action, so far as movement is concerned, is similiar to what is so well known in the squid, and differs from the movement of *Solenomya* only in direction (2). Here the movement is posterior, in *Solenomya* the movement is anterior. Here the water is admitted through the siphons, and possibly also around the foot, and then, with the siphons closed, the water is thrown from the anterior end of the animal. In *Solenomya* the water is admitted around the foot, which opening is then closed and the water is thrown through the posterior opening. The method of forming the jet is quite the same in both animals. In both forms the same organs are used, but in *Solenomya* more use is made of the mantle margins and less of the retraction of the foot than is the case in this form.

Throwing strong jets of water from the siphons must aid lamellibranchs in keeping their mantle chambers clean. Some forms need to throw more powerful jets than others because the conditions under which they live demand it. A diversion of this use is apparently to be seen in the forms that swim either by clapping the shell valves together, as in the case of *Pecten* (3), or by the more complicated method used by *Solenomya* and the form under consideration. It is likely that the jets thrown by this form are of very secondary importance, so far as swimming is concerned, and that their chief function is to aid the animal in burrowing. This is indicated by the fact that the jets are thrown from the anterior end of the shell, while in forms that use them for cleaning the mantle chamber only, they are thrown from the siphons.

Leaping. — Leaping may consist simply of a sudden, powerful protrusion of the foot, in which case the animal generally turns so as to lie somewhat nearly on its dorsal margin and catches the tip of its foot in the mud as it is protruded. The shell is thus thrown posteriorly. Generally, however, the foot is bent back under the shell, which is turned partly over towards its dorsal margin (Fig. 3) and is then suddenly made rigid with the result that it straightens out with great rapidity. This may result in projecting the animal backward, or in certain cases the foot may catch so as to turn the shell more or less completely end for end. Leaping movements are usually rapidly repeated several times when they are once begun. In many ways they resemble similar movements in *Yoldia* and *Solenomya* (2), but the foot of this form is so much longer that the impression of much greater activity is left with the observer.

The perfection of the movements of burrowing by a form that lives in the mud, so it may be able to escape its enemies, is of so much importance as to need no comment. When combined with sense organs that give immediate information of the presence of enemies, and with protective coloration that hides it from its enemies until they shall have given it warning, the rapid burrowing movements form a striking adaptation.

The uses of the swimming and leaping movements are not quite so evident. Small razor-shell clams have been taken in tow nets at the surface of the sea. The ability to swim is, then, sufficient to make it possible for the young specimens, at least, to change their positions after settling to the bottom, and after the larval locomotor organ, the velum, has been lost. If the first location does not offer the necessary food or bottom conditions, it is possible to move. Although the machinery for this is rather clumsy, and was primarily designed for another purpose, that of burrowing, it might be the deciding factor in the struggle of life. The fact that young specimens may be taken at the surface of the water, even the fact that the animals are able to swim at all, indicates that they probably occasionally change their positions. The statement made by Woodward (8) that the animals never voluntarily leave their burrows seems doubtful. They certainly do leave the mud when they are about to die, and there is no reason to believe that they might not voluntarily move from one place to another should occasion require.

Leaping may aid the animals in getting free from certain kinds of bottom or even occasionally in escaping enemies, should they be removed from the mud in any manner. Not infrequently specimens that have been swimming might become lodged so they could not burrow without changing their positions, and then the leaping movements would be of advantage.

Summary.

The animal is very active, burrows with great rapidity, and may also swim and leap.

In burrowing, the foot is worked into the mud as it is protruded, the end is then swelled into a knob, and by its sudden withdrawal the shell is drawn to the position of the anchored end of the foot. Simultaneous with the retraction of the foot, a strong jet of water is thrown from the anterior end of the shell, so the mud is softened or even washed away as the shell descends, an action similar to that of some of the modern pile drivers. A collar, formed by the anterior end of the mantle, surrounds the foot and acts as a scraper that prevents mud from being drawn into the shell with the foot.

The animal is able to swim by throwing jets of water from the anterior end of the shell, thus progressing backward by a series of jerks.

By the uncommon activity of the foot the animal is able to throw itself about on the bottom.

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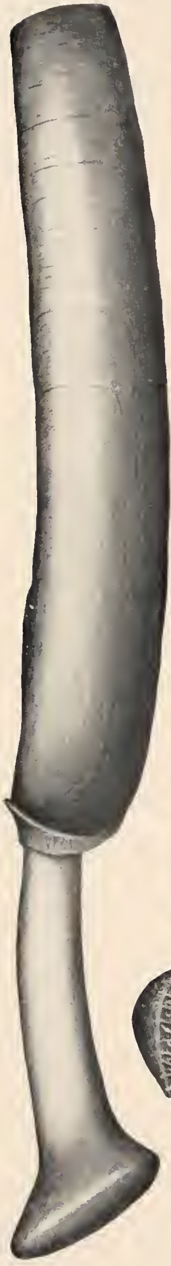

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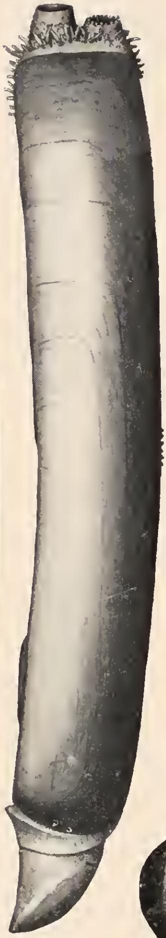

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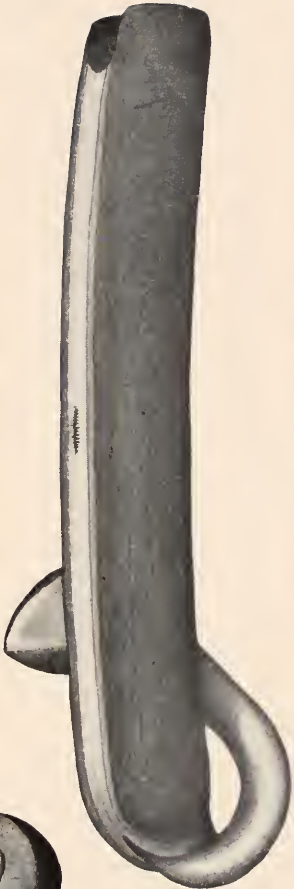

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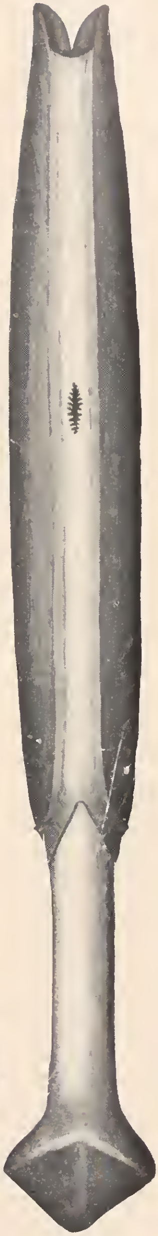

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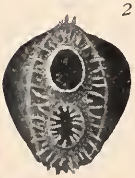

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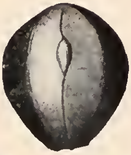

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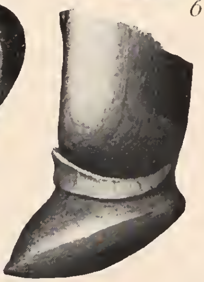

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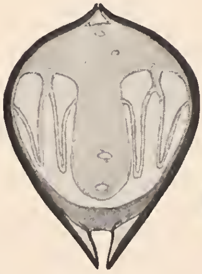

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Explanation of Plate II.

Fig. 1. A specimen with the foot extended and the end of the foot swelled, the instant previous to withdrawal. Drawn with the aid of an instantaneous photograph made by Mr. J. G. Hubbard of a specimen held with the anterior end down, and stimulated into activity. Natural size.

Fig. 2. A specimen showing the usual position assumed in a dish of sea-water. The siphons may be extended more than is shown in this figure. Drawn from observations. Natural size.

Fig. 3. A specimen showing the position assumed just before leaping. Drawn from observations. Natural size.

Fig. 4. Ventral view of a specimen that has the foot extended to the position shown in Fig. 1. Drawn from observations. Natural size.

Fig. 5. Direct view of the posterior end of an animal that has the siphons extended. Drawn from observations. Natural size.

Fig. 6. Direct view of the anterior end of a specimen that had withdrawn all but the tip of the foot, showing the adjustment of the collar. Drawn from observations. Natural size.

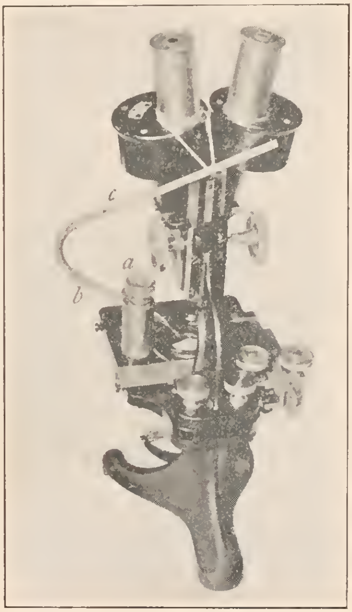
Fig. 7. Anterior end of a specimen showing the position and shape of the foot at the end of a burrowing movement. Drawn from observations. Natural size.

Fig. 8. Diagrammatic cross section of an animal, taken just posterior to the ventral opening in the mantle, to show how the mantle chamber is diminished by the contraction of the united mantle margins. Twice natural size.

EXPERIMENTS ON THE EGGS OF CHÆTOPTERUS AND ASTERIAS IN WHICH THE CHROMATIN WAS REMOVED.

J. F. McCLENDON.

Last July and August, while in the U. S. Bureau of Fisheries Laboratory at Wood's Hole, Mass., I devised an apparatus for removing blastomeres from ascidian eggs, but as the chorion of these eggs was too tough for a successful operation, I tried to use the apparatus for other purposes. I found that with it I could remove parts of the unsegmented eggs of echinoderms and annelids, and, in the few weeks at my disposal, performed the experiments mentioned below.



The Apparatus.

To a Greenough binocular stand (see accompanying figure) I attached a Spencer mechanical stage supplied with a fine adjustment screw (*a*) made by Wieback and Pietzsch, Philadelphia. The addition of this screw allowed movement in three dimensions, and the fine adjustment (*a*) carried a tube (*b*) drawn to a capillary opening at the lower end, and with the upper end attached by a rubber tube to the glass tube (*c*), which I held in my mouth during the operation. The lenses used were Zeiss binocular objective a_3 and a pair of Zeiss orthomorphic oculars, 4, giving a magnification of 65 diameters. Eggs were placed in 2–3 mm. depth of sea water in a glass dish on the stage of the microscope and the capillary end of the tube (*b*) inserted into the water above them and allowed to remain until the water rose in it as high as it would by capillarity. The dish was moved until an egg of the most favorable orientation was brought in the center of the field. The capillary tube was then brought against the proper spot on the egg and a portion of the egg sucked away through the tube (*c*) which was held in the mouth. Immediately the operated egg was lifted from the dish by means of a capillary pipette just large enough to admit it easily, and placed in a watch glass half full of sea water. This capillary pipette does not need a bulb, as water enters it by capillarity. The size of the capillary end oi the tube (*b*) was determined for the egg of each species by trial Some parts of the mechanical stage were an unnecessary hindrance and were removed.

Experiments on the Eggs of Chætopterus Pergamentaceus Cuvier.

Mead ('98^2) and J. Loeb ('01) found that differentiation went on in the unfertilized egg of *Chætopterus* in solutions of KCl, and F. R. Lillie showed that this differentiation bears a semblance to the normal development save that cell division is usually suppressed.

I removed the chromosomes during the maturation divisions of the unfertilized egg in KCl solutions by sucking away the part of the egg immediately under the first polar body, in order to determine the effect of the chromatin on differentiation. A small percentage of eggs were so injured by the operation that they remained inactive until disintegration. Others, however, performed those "amœboid" movements which Loeb mistook for cleavage, just as in operated eggs. Further than this no changes were visible externally, but sections showed that irregular flowing of substances had taken place within the egg. None of the operated eggs developed cilia, and we might conclude that the chromatin is necessary for the formation of cilia, a function that has been attributed to the archoplasm. But as the centrosomes were probably removed with the chromosomes, and as the element of injury could not be excluded, this negative evidence should not be considered conclusive. Nearly one hundred eggs were operated on, and, although I do not think it advisable to record the individual experiments, I will state the precautions against error that were used:

The majority of the *Chætopterus* females were gathered by me, or under my direct supervision, and were never placed in the same vessel with males. Each female was washed in a stream of fresh water before eggs were removed from it. Except during two days no males were allowed in the same laboratory with the females. One check of eggs in sea-water and another in sea-water to which KCl had been added, were kept to each experiment. None in the check in plain sea-water developed, while many in the KCl sea-water developed cilia. In each lot of eggs the operations were continued from the first appearance of the first polar body to the formation of the second polar body, and sometimes a few minutes later. Some eggs were fixed and sectioned immediately after the operation, others at later intervals up to twenty-four hours, and in this way was determined whether any chromatin had been left in the egg.

Experiments on the Eggs of Asterias Forbesii.

Eggs from which the chromosomes were removed and eggs from which the whole nucleus was removed were fertilized with sperm of the same species. These operated eggs did not show any differences from the normal save in number of chromosomes, in increased tendency toward polyspermy and in increased mortality. This last prevented my ascertaining whether any differences would appear in the later development.

An attempt was made to remove some of the chromosomes during the first cleavage, but the spindle was so much more viscid than the surrounding yolk that no part of it could be sucked out without removing all of it. In some sections the spindle was shown pulled to the surface of the egg but not very much distorted.

Eggs from which chromosomes were removed were mixed with sperm of a species of *Synapta*, but did not show signs of development save for the separation of the "fertilization membrane" from the egg.

In the experiments on the *Asterias* egg the following precautions were used: The starfish were washed in a strong stream of fresh water before removal of the sexual organs, and after a male had been opened my instruments and hands were washed in like manner, to remove all spermatozoa. A check of unfertilized eggs was always kept, and in case some eggs developed in the check, the material was thrown out. In a number of experiments the eggs were fixed and sectioned as a further check.

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THE CONUS ARTERIOSUS IN TARPON ATLANTICUS (CUVIER & VALENCIENNES).

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The tubular prolongation of the arterial end of the heart furnished with numerous valves, and known as the conus arteriosus, is one of the characteristic features of elasmobranchs and ganoids. In *Amia calva*, the conus arteriosus is relatively shorter than in other ganoids, and its valves are reduced to three transversely arranged tiers. It may be said that the absence of the conus arteriosus as a separate structure is a characteristic of the teleostean heart, and that this fact is emphasized by a few recorded exceptions, all of which occur in teleostean families more or less closely related to *Amia*.

Among these exceptional teleosts only one has been hitherto described, which possesses more than one tier of conus valves, this is *Butirinus* (*Albula*) which has two, and to it may now be added *Tarpon atlanticus*.

The heart from which the following description is taken, was sent to me by Mr. Charles H. Townsend, director of the New York Aquarium. It comes from a specimen 5 feet 4 inches in length.[1] I take this opportunity of thanking Mr. Townsend for his courtesy in sending this heart, also an entire *Tarpon*, 4 feet 4 inches long, the heart of which is shown in Fig. 3.

The conus of *Tarpon atlanticus* resembles that of *Amia calva* in form, but differs from it in being proportionately smaller, in having two tiers of valves instead of three, and in appearing to have been driven into the heart towards the apex, so that, instead of projecting freely from the ventricle, as in *Amia*, it is more or less buried in the latter.

In the natural position, the conus of *Tarpon* is a horizontally placed longitudinal tube, elliptical in transverse section. The longest diameter of the ellipse is dorso-ventral, and measures 16 mm., the shortest measures 11 mm., and is transverse. The conus wall is slightly under 2 mm. in thickness. The total length of the conus varies according to the site of measurement, at the mid-dorsal and mid-ventral lines it measures 8 mm., laterally its measurement increases, until at the mid-lateral line on either side it becomes 10 mm.

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[1] Measurements include caudal fin.

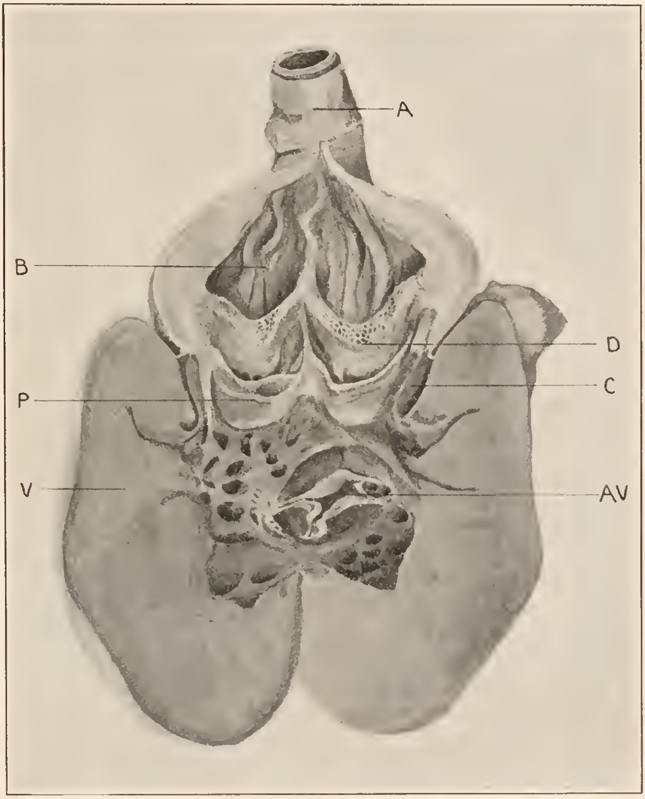

Fig. 1. The heart has been opened by a mid-ventral sagittal incision, and the parts widely separated (natural size). *A*, aorta; *AV*, atrio-ventricular valve; *B*, bulbus arteriosus; *C*, conus arteriosus; *D*, left distal conus valve; *P*, right proximal conus valve; *V*, wall of ventricle.

In order to compare the relative lengths of conus and ventricle in *Tarpon* and *Amia*, the length of the ventricle has been measured by plunging a needle into the apex of the ventricle, in such a direction that its point emerges where the ventricle and conus blend. In this particular *Tarpon*, the ventricle thus measured is 41 mm. long, and taking 9 mm. as the average length of the conus, the proportion of the conus length to ventricle length, becomes 1 to 4.5. Six *Amia* hearts measured in the same way yield an average proportion of conus to ventricle of 1 to 1.76.

The exterior of the conus presents relations which differ in different regions. At the mid-lateral line, and ventral to this, the ventricle covers the conus completely. Dorsal to the mid-lateral line, the ventricle recedes rapidly, so as only to overlap the conus for a short distance on either side; in the interval, the conus is incompletely covered by the atrium. The area uncovered by ventricle and atrium measured back from the bulbus, is about 3 mm. in the midline, and lateral to this about 4 mm., it is covered by visceral pericardium. (These relations are indicated in Fig. 2.)

The conus is everywhere overlaid by a distinct layer of loose connective tissue, which separates it from the structures which cover it, and renders its outline very distinct in sections of the heart. Owing to the looseness of its connection with neighboring structures, the entire conus is easily exposed from the outside by incising the pericardium at the base of the bulbus, and stripping it away from the adjacent parts of the ventricle and atrium.

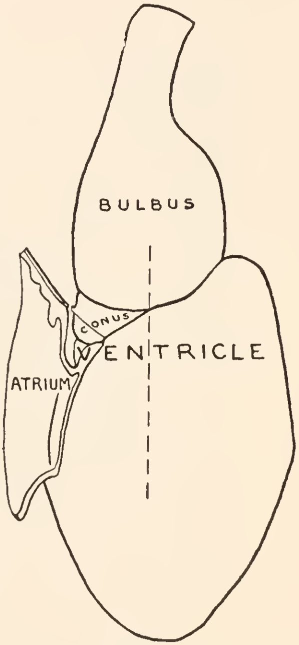

FIG. 2. Diagrammatic right lateral surface-view of bulbus, conus and ventricle. The atrium is represented as incised mesially, and the right half removed (natural size). The line across the conus indicates the site of reflection of visceral pericardium on to the atrium. The broken line indicates the site section in Fig. 3.

The conus valves are disposed in two transverse rows. Each row consists of a right and left cusp symmetrically placed with regard to the median dorso-ventral plane of the conus. Seen rom the lumen of the heart (as in Fig. 1) the valve cusps of the proximal[1] and distal[1] rows appear to be approximately equal in size, this however is far from being the case, those of the distal row having a capacity far exceeding that of the proximal. The proximal valves are extremely fleshy at their attached margins, and shade rapidly into a thin semilunar area near the free edge; the edge itself is marked by a cord-like thickening, and is quite unattached, except at either end, where, having blended with the corresponding extremity of the other cusp, it is attached dorsally and ventrally to the mid-line of the bulbus a short distance beyond the conus.

The distal valves are not so fleshy as the proximal, and the marginal semilunar area is very thin and profusely perforated. The margins are free except at their extremities, the dorsal ends of the right and left valves blend at the mid-longitudinal region of the bulbus, and become continuous with an elastic cord, the other end of which is attached to the dorsal bulbus wall at its distal extremity. The ventral extremities of the distal valves blend at their point of attachment in the mid-line at the junction of the proximal and middle thirds of the ventral bulbus wall.

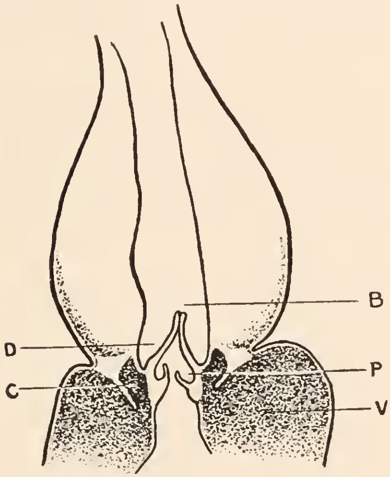

FIG. 3. Ventral face of a frontal section of the heart from the smaller, *Tarpon* its approximate source is indicated by the broken line in Fig. 3 ($\times \frac{4}{3}$). *B*, lumen of bulbus arteriosus; *C*, conus arteriosus; *D*, cavity of right distal conus valve; *P*, cavity of left proximal conus valve; *V*, wall of ventricle.

That the capacity of the distal valves is much greater than that of the proximal, is shown by the fact that a probe can be passed 11 mm. into the former, and only 3 mm. into the latter. To illustrate this point, a frontal section passing approximately through the mid-lateral line of another heart is shown in Fig. 3.

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[1] The terms proximal and distal are used with regard to the ventricle.

The conus in *Tarpon* appears to differ from that of *Butirinus* (*Albula*) described by Boas ('80) in that it is less overlapped by the bulbus arteriosus, and more deeply buried in the ventricle, also in that it shows no diminution in length dorsally, as compared to the ventral measurement. The two subsidiary valves between the larger ones of the proximal row in *Albula* do not occur in *Tarpon.*

The sinu-atrial valves are two, with strong tensor muscles. There are four atrio-ventricular valves of which two are of large size, and two somewhat smaller. The hepatic vein, at its junction with the sinus venosus, is of almost cartilaginous rigidity, the size of the orifice is reduced by a thin fold of intima on either side, these almost meet mesially to convert the circular orifice into a vertical slit. The folds of intima appear to have no valvular action.

It is singular that since the appearance of Stannius's paper ('46) *Albula* should have enjoyed the reputation of being the only teleost provided with a conus having two rows of valves; whether the heart of *Megalops cyprinoides* will also prove to have more than one row of valves is an open question. So far as I am aware a description has not been recorded.

Of the other fishes showing evidence of near relationship to *Amia* the following have been examined with a negative result:

Elops saurus by J. Mueller ('46), *Hyodon* by Mueller ('46) and Boas ('80), *Osteoglossum* by Mueller ('46) and Boas ('80), *Notopterus* by Boas ('80), *Mormyrops* by Mueller ('46). I have also examined *Elops saurus* (for a specimen of which I hereby beg to thank the authorities of the U. S. National Museum) *Hyodon tergisus* and *Notopterus borniensis.*

The original opinion of Gegenbaur ('66) which has been restated and amplified by Hoyer ('00) that the conus, although it has ceased to exist as a separate structure in the ordinary teleost heart, is represented by the portion of the myocardium adjacent to the aortic valves, is well illustrated by the conus relations in *Tarpon.* One has only to imagine the connective tissue layer between the exterior of the conus and the ventricle to have disappeared, allowing the conus muscle to be merged into the general myocardium, and the transition is complete; the relation of the myocardium to the distal valve will be similar to that generally found in teleosts. An interesting transitional stage can be seen in the heart of *Dorosoma cepedianum*, where there is an extremely thin but distinct streak of connective tissue projecting into the myocardium for sufficient distance to clearly separate the areas of original conus and original ventricle.

Philadelphia,
October, 1906.

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SOME SILKWORM MOTH REFLEXES.

VERNON L. KELLOGG.

Silkworm moths, *Bombyx mori*, are sexually mature and eager to mate immediately on issuing from the pupal cocoon. They take no food (their mouth parts are atrophied), they do not fly, they are unresponsive to light; their whole behavior, in fact, is determined by their response to the mating and egg-laying instincts. We have thus an animal of considerable complexity of organization, belonging to a group of organisms well advanced in the animal scale, in a most simple state for experimentation.

The female moth, nearly immobile, protrudes a paired scent-organ from the hindmost abdominal segment, and the male, walking nervously about and fluttering its useless wings, soon finds the female by virtue of its chemotactic response to the emanating odor. Males find the females exclusively by this response, but orient themselves for copulation (after reaching the female) by contact. When two males accidentally come into contact in their moving about they try persistently to copulate.

A male with antennæ intact, but with eyes blackened, finds females immediately and with just as much precision as those with eyes unblackened. A male with antennæ off and eyes unblackened does not find females unless by accident in its aimless moving about. But if a male with antennæ off does come into contact, by chance, with a female it always (or nearly so) readily and immediately mates. The male is not excited before touching the female, but is immediately and strongly so after coming in contact with her. Males with antennæ on become strongly excited when a female is brought within several inches of them.

The protruded scent-glands of the female are withdrawn into the body immediately on her being touched by a male. If the scent-glands are cut off and put wholly apart from the female, males are as strongly attracted to these isolated scent-glands as they are to unmutilated females; on the contrary they are not at all attracted to the mutilated females. If the cut-out scent-glands are put by the side of and but a little apart from the female from which they are taken, the males always neglect the near-by live female and go directly to the scent-glands. Males attracted to the isolated scent-glands remain by them persistently trying to copulate with them, moving excitedly around and around them and over and over them with the external genitalia vainly trying to seize them.

The behavior of males with the antenna of only one side removed is striking. A male with left antenna off when within three or four inches of a female (with protruded scent-glands) becomes strongly excited and moves energetically around in repeated circles to the right, or rather in a flat spiral thus getting (usually) gradually nearer and nearer the female and finally coming into contact with her, when he is immediately controlled by the contact stimulus. A male with right antenna off circles or spirals to the left. It is a curious sight to see two males with right and left antenna off, respectively, circling violently about in opposite directions when the immobile female a few inches removed protrudes her scent-glands. This behavior is quite in accordance with Loeb's explanation of the forward movement of bilaterally symmetrical animals.

The results of all the experiments tried show how rigorously the male moths are controlled by the scent attraction (chemotropism) and how absolutely dependent mating (the one adult performance of the males) is on this reaction. If we can find specialized animals in a condition where all attractions and repulsions (stimuli) but one are eliminated we may readily perceive the rigorous control exercised by this remaining one. We are, unfortunately, in the general circumstances of animal life too much limited to the use of very simply organized animals for reaction and reflex experimentation. This tends to make it difficult to carry over to the behavior of complexly organized animals the physico-chemical interpretation which is steadily gaining ground as the key to the understanding of the springs and character of the behavior of the simplest organisms. But where the complex stimuli and reactions that determine the behavior of complexly organized forms can be isolated and studied the inevitableness of much of this behavior can be recognized.

Reflexes of Moths Without Cephalic and Thoracic Ganglia.—

A number of experiments was made to determine the need, or absence of need, of the principal ganglia of the central nervous system in the performance of the two chief reflexes in the silkworm moth's life, viz., mating and egg-laying.

Males mate with headless females, and the headless females, after mating, lay a few eggs which develop normally, that is become fertilized by the release of spermatozoa from the spermatheca in the female's body, are oviposited by the repeated extrusion and retraction of the ovipositor, and make the usual color changes (from yellow to cherry-red and then to lead-gray) incidental to normal development. But in no case did a headless female lay her full complement of eggs, in fact in no case were more than a score of eggs laid (the normal number is from 200 to 350). Headless females (and headless males) usually live as long as unmutilated individuals, *i. e.*, from a week to two weeks.

Females with head and thorax cut off (and even part of the abdomen) can be mated with by males, and this fractional part of the female can fertilize and oviposit a few eggs which begin normal development. In one case 10 eggs, of which 8 are now normally developing were oviposited by such an impregnated part of female abdomen, this abdominal relict remaining alive (!), *i. e.*, flexible and responsive to stimulus and capable of extruding the ovipositor and laying eggs, for forty hours.

Males with head removed cannot find females, nor can they mate if placed in contact with them. When the head or head and prothorax of a male is cut off immediately after the male and female are *in copulo* the female, although uninjured, lays no eggs. If heads of both males and females *in copulo* are removed no eggs are laid although both moths remain alive usually as long as do unmutilated individuals.

A silkworm moth can maintain itself right side up with antennæ off or with antennæ off and eyes blackened, but with head off one position seems indistinguishable from another to it, *i. e.*, it lies on one side or the other, on the venter or dorsum equally willingly. The organs of equilibrium are not on the antennæ, then, but are lost when the rest of the head is removed.

Stanford University, Calif.,
October 15, 1906.

AN ABNORMAL CESTODE PROGLOTTID.[1]

EDWIN LINTON.

In the summer of 1905, while engaged in work for the Bureau of Fisheries at the Woods Hole Laboratory I found an interesting cestode abnormality, a description of which is here given.

Among a lot of free segments of the cestode *Calyptrobothrium occidentale* Linton from the torpedo, I noticed one which had two reproductive apertures upon one of the lateral margins. Upon flattening this segment under a cover-glass it was found to be double. The reduplication can be best explained in general by saying that it is such as would be formed if two normal proglottids were to grow together by their anterior ends.

The specimen was fixed over a flame, and after the customary hardening was stained with borax carmine and mounted in balsam. Fig. 1 was sketched from the mounted specimen. The specimen measured 4 millimeters in length and 2 millimeters in breadth.

As may be seen by an examination of the figure the specimen would give rise to two complete proglottids if it were transversely bisected midway between the two reproductive apertures. At the opposite ends will be seen the lobed ovaries (*o*, *o*) arranged symmetrically on either side of the median line, with the shell gland (*sg*, *sg*) between the two lateral masses of the ovaries and toward the extremity of the proglottid.

The reproductive apertures are on the same lateral margin, each being approximately not far from one third the total length of the double segment from one end.

Each of the two parts, considered by itself is a normal segment, so far as the general arrangement of the various organs is concerned, except that there is relatively less space taken up by the testes (*t*) which are massed together in the median region and possessed in common by the two component parts of the double segment.

The vitelline glands (*vg*, *vg*) are continuous along the margin

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[1] Published by permission of the Bureau of Fisheries.

opposite the reproductive apertures. On the opposite margin a vitelline mass separates the two vaginæ and, like the testes, belongs to both components of the double segment.

There is no line of demarcation between the two components. The margins are continuous and smooth everywhere except between the reproductive apertures where the minute serrations, which are characteristic features of younger portions of the strobile, are seen. These serrations are inclined towards the extremity of the smaller part, and may be taken as belonging to that part.

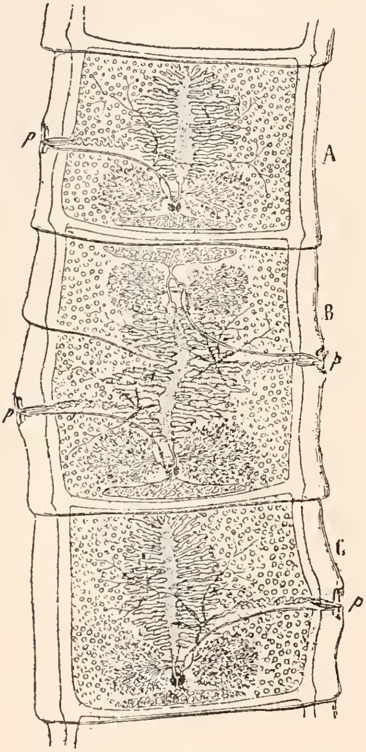

FIG. 2. After Blanchard. Abnormality of genital organs of *Tænia saginata*. *A*, *C*, normal segment; *B*, abnormal segment.

There is no indication as to which is the older of the two parts, except a slight difference in size. Evidently the reversal took place very soon after the primary segment was formed. Although the segment was not seen attached to its strobile it is not conceivable that the abnormal condition was assumed after separation from the strobile.

It is to be noted that there is a reversal in a dorso-ventral direction also, the vagina and oviduct lying above the uterus in one part and below the uterus in the other. Or, to state this comparison in another way, if the specimen were folded together on a hinge-line crossing transversely between the vaginæ the two components would not be symmetrical to the plane of their apposed faces, but one would correspond in the position and arrangement of the parts to the other.

Abnormalities in cestodes are of common occurrence and the

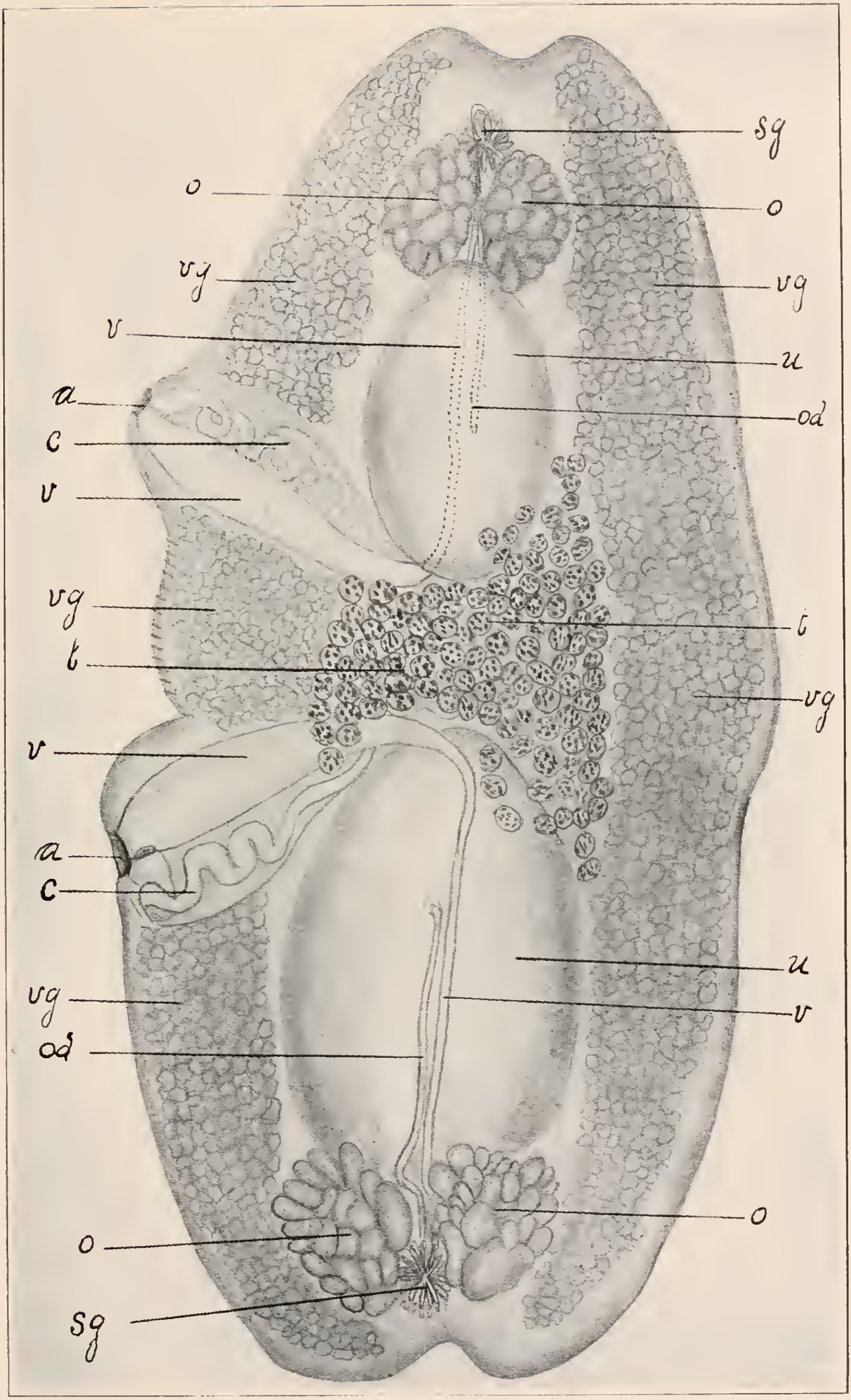
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FIG. 1 Abnormal segment of *Calyptrobothrium occidentale* Linton from the torpedo. Actual length 4 millimeters, *a*, reproductive aperture; *sg*, shell gland; *c*, cirrus; *t*, testes; *o*, ovary; *u*, uterus; *od*, oviduct; *v*, vagina; *vg*, vitelline gland.

literature of the subject is considerable. The only case, however, which at all resembles the subject of this sketch, that I find in the literature of the subject, to which I have access, is the one recorded by Blanchard for *Tænia saginata* (*Bulletin de la Societé Zoologique de France*, 1890, XV., 166–168, and reprinted in *Progrès Médical*, July, 1894).

My friend Dr. Stiles informs me that, while he has repeatedly found abnormalities in cestode segments, he has not made any record of any cases such as is described in this paper.

On account of the unique character of this abnormality, therefore, and for purposes of comparison I reproduce Blanchard's figure (Fig. 2).

In Blanchard's specimen the ovaries and vitelline glands are at opposite ends of the abnormal segment; the testes are continuous along the lateral margins; the reproductive apertures are on opposite lateral margins; the uterus is common to both parts. At about one third of the length from the anterior end there is a transverse incision which reaches to the median line, and is followed on its side by a perfectly normal segment. If this incision were to extend somewhat diagonally across, so as to reach the other margin behind the reproductive aperture, there would then be a single inverted segment intercalated between two normal segments.

It should be stated here that the abundance of material found in the summer of 1905 furnishes additional data, which will make it necessary to separate the two varieties noted in the original description (see Bulletin of the United States Fish Commission for 1899, p. 298) into two distinct species.

The abnormal segment belongs to the larger variety which will retain the name *C. occidentale*.

Washington and Jefferson College,
December 1, 1906.

OBSERVATIONS ON THE YOUNG OF RANATRA QUADRIDENTATA STAL.

S. J. HOLMES.

In two previously published papers[1] I have described certain features of the behavior of the water scorpion, *Ranatra*, and as opportunity presented itself this last spring of obtaining young *Ranatras* in abundance attention was devoted to a comparison of the behavior of the young and the mature forms. The various stages in the metamorphosis of the nymph were followed, but as these have recently been described by Torre-Bueno[2] the observations made on this subject are omitted.

Ranatra lays its eggs in the spring commonly in the stems of aquatic plants or floating pieces of wood. The eggs are inserted in the material containing them so that they are nearly buried. They are of cylindrical form, rounded at either end, and provided at the outer end with a pair of slender filaments of uncertain function. When one sees a *Ranatra* which has recently emerged from the egg he cannot but be surprised that the young insect should have been enclosed in so small a receptacle. The body of the young *Ranatra* is as broad as the egg and over twice as long. The length of the young, measured from the end of the proboscis to the tip of the breathing tube is frequently 8 mm., while the egg itself is only about 3 mm.

The young in general appearance closely resemble the adult form. The prothorax, however, is relatively shorter, the wings are entirely absent, and the breathing tube, or what functions as such,[3] is relatively short, being about one fourth of the length of the body, while in the adult it is about four fifths the length of the body. At first the young are very soft. The slender legs bend with the greatest ease, and if the insect is taken out of the water it is unable to support itself and wriggles about helplessly. Its integument rapidly hardens, however, and in the course of a few hours it is able to move around out of water as well as an older individual.

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[1] Holmes, "The Reactions of Ranatra to Light," *Jour. Comparative Neurology and Psychology*, Vol. 15, p. 305. "Death Feigning in Ranatra," *l. c.*, Vol. 16, p. 200.

[2] Torre-Bueno, *Canadian Entomologist*, Vol. 38, p. 242, 1906.

[3] Torre-Bueno, *l. c.*, has shown that the so-called breathing tube of the young nymph differs essentially in structure from that of the adult insect.

When first hatched the young are pale in color, but they soon become much darker, many specimens becoming quite dark in less than a day.

The manner of walking, swimming, turning over when placed on the back, and the attitudes assumed when resting at the surface of the water, or in contact with objects below the surface, are very nearly the same as in the mature insect. Soon after hatching the young *Ranatras* take up a position at the surface of the water with the tip of the breathing tube just projecting through the surface film and the body inclined obliquely downward. The second and third pairs of legs are held in a sprawled out position, while the first pair is held in front, and bent upward at the middle, with the claw held open. The position is one of readiness for quickly seizing any small object that passes within reach. Young *Ranatras* are remarkably active in the capture of prey. Any small object that strikes the outstretched arms is grabbed at with surprising quickness. If a small insect or crustacean is seized, it is drawn towards the mouth, usually with the assistance of the other arm, and the proboscis is moved about over it in the endeavor to find a soft spot through which it can penetrate. When this is found the juices of the body are gradually sucked out and the rest of the prey rejected. Young *Ranatras* will seize and suck out almost any animal not of too large size. I observed one not a day old deftly catch a small ostracod that happened to swim against one of its outstretched arms. The ostracod tightly closed the valves of its shell, but the *Ranatra* turned it over and over, exploring all sides of it with the tip of its proboscis and endeavoring in vain to force an entrance between the valves. After the round smooth ostracod was rolled about for several minutes it slipped from the grasp of its captor and swam away.

Ranatras may readily be fed by seizing a small organism in a pair of pincers and carefully bringing it up to them. Animals as large as themselves are successfully coped with. Large *Hyalel-*

las are seized and sucked out without much difficulty. While mature *Ranatras* will live together peaceably for a long time the young readily attack and devour one another. If several young are kept in the same dish it will be found that, in the course of a few days, a majority of them will have fallen victims to a few successful combatants. If a young *Ranatra* seizes another near the middle of the body it is usually able to bring its victim up to its proboscis, the tip of which is moved about in search of a soft spot in the armor of the unfortunate individual, whose blood is then deliberately sucked out notwithstanding the creature's struggles. Often a *Ranatra* is seized by one of its legs. This is usually not resented until its captor, after pushing the tip of its proboscis along the leg until it finds one of the joints, begins to insert its piercing stylets through the soft integument, when a vigorous struggle ensues.

Young *Ranatras* are exceedingly voracious creatures, as they will kill and suck out several insects as large as themselves in the course of a day. Their food consists mainly of small swimming forms, chiefly small crustaceans and insects, which come near the surface of the water. Like the adults, they are very efficient enemies of the larvæ of mosquitoes. They do not pursue their prey, and they seldom catch forms that keep in close contact with solid objects. They are like so many traps set ready to seize anything that comes in contact with them. Often, however, an object is grabbed at if it passes near a *Ranatra* without coming into actual contact with it. This action is probably a response to the impact of the water. If a *Ranatra* is hungry, touching the surface film with a needle near the insect will often cause it to grab about wildly in the effort to seize whatever may have caused the disturbance. An object of too large or threatening appearance causes the young *Ranatra* to jerk back its first pair of legs, but there are no efforts to swim away from danger. When this reaction occurs the insect cannot be induced to take food for some time.

The reactions of young *Ranatras* to light are not nearly so vigorous and decided as those of the adult. A feeble positive phototaxis is manifested the first day after hatching and increases gradually as the insect grows older. Individuals a week old are very often found swimming on the side of the dish towards the light; if the dish is turned about they quickly swim back again to the light side. When out of water they are comparatively irresponsive to light — a fact in marked contrast to the behavior of the mature insects. Movements of the head in response to changes in the position of the light, which are so pronounced in the adults are manifested in the young of a week old or even less, but they are not very pronounced. When out of water the young could not be induced to walk toward the light or respond to it in any other way than by making rather feeble movements of the head. While contact stimuli applied to the mature insects when in the water cause a negative phototaxis, they failed in the forms experimented with to produce this effect in the young.

The death feigning of young *Ranatras* is not so decided or prolonged as in older specimens, and it also differs in certain other particulars. Young *Ranatras* when taken out of the water and laid on a table frequently become immobile in whatever position they may happen to lie. Neither in the young nor the mature form do the appendages assume any definite position such as they do in the death feint of many other insects. The feint is shown during the first day of free life. The muscular system gives evidence of a certain degree of rigidity, but owing to the flexibility of the appendages this is not so clearly manifested as in somewhat older individuals.

In specimens five days old the death feint is more decided. Several specimens of this age were taken out of a dish and laid on a table. Immediately they all became immobile. They could be picked up by one of the slender legs and held out without causing a bend in any of the joints, thus showing that the muscles were in a state of extreme contraction. Many specimens would endure considerable handling and poking about without making any response. In some cases such treatment would bring them quickly out of the feint, and all the forms experimented with were brought out of it by more prolonged stimulation. In this respect the young differ from the mature insects which will endure a great deal of maltreatment without making any response.

In specimens coming out of the death feint handling or rubbing them with a dry camel's hair brush produced in some cases a resumption of the feint; in others it had no effect. This, too, is different from the characteristic reaction of the adults which, when they awaken from the feint, can readily be caused to resume feigning many times in succession by handling or gently stroking them. And if an adult is picked up while feigning it is rarely brought out of its feint by this means.

It is a curious fact that while the mature *Ranatra* will endure all sorts of maltreatment during the death feint, even suffering its legs to be cut off one by one or its body cut in two without the least response, the moment the insect is placed in the water the death feint entirely disappears. There is no way in which the feint can be terminated so quickly and completely as by this means. Nor is it possible by any sort of manipulation to cause the insect to feign death so long as it is in the water. It is certainly remarkable that an insect that will feign, it may be for hours, with its muscular system tense so that all its appendages are perfectly stiff should so completely and suddenly change its behavior when it is placed in another medium.

In *Ranatras* of five days old or less the death feint often persists for a time after they are placed in water. They do not, as a rule, swim directly away, as the adults do, but frequently remain motionless and apparently still for several seconds, or in some cases for over a minute. When in the air the duration of the death feint of the young is increased if they are kept wet. Specimens that refuse to feign when rubbed with a dry camel's hair brush can commonly be made to do so by rubbing them with a brush dipped in water. And when specimens cannot be induced to feign by this means they can generally be thrown into a feint by dipping them into water and then leaving them on the table. Dryness produces in the young a restlessness that is not shown by the adult, — a result not improbably due to their less ability to withstand lack of water.

The young, like the mature forms, can be cut in two while in the death feint without causing any response. A specimen which was cut across near the middle of the body showed no movement in either piece; the legs were rigid, but after being poked about for some time they began to move. Handling the anterior piece failed to cause it to feign again, but when it was dipped into the water and placed back on the table it feigned for several minutes, the legs giving the same signs of muscular rigidity as before. Several times it came out of its feint and as many times it was caused to resume feigning by dipping it into water and placing it on the table. Contact with a wet camel's hair brush would readily cause it to feign, but a dry brush would produce no feint, or but a very short one. Other experiments gave very similar results.

It is difficult to understand how the death feint in *Ranatra* can be of much value to it. While the European *Ranatra linearis* has been known to fly to lights at night (a rare occurrence apparently) I have been unable to obtain any evidence of such a habit in any of our American species. In fact *Ranatra* very seldom leaves the water of its own accord on account of any sort of inducement, and one is.therefore strongly inclined to believe that the death feint which is manifested only when the insect is in the air is rather an incidental result of certain physiological peculiarities of the organism than an instinct which has been built up by natural selection for the benefit of the species. We must adopt such a view, I think, regarding hypnotism in the higher animals and in man ; for what selective value can it be to a species, such as our own for instance, to possess the capacity of being thrown into the hypnotic state ? The instinct of feigning death is of unquestionable service to many forms, and it is possible that in rare instances it may have proven of selective value to *Ranatra*, but it is open to serious question if the instinct in this form has been evolved because of its importance as a means of protection.

The strong and at times almost violent positive phototaxis which *Ranatra* exhibits presents another problem of the same kind. Certain of the most striking features of this reaction are manifested only when the insect is out of water. As a rule *Ranatra* inhabits more or less shaded retreats among submerged grass or weeds near the water's edge. It is kept in such situations, partly through the direct effect of its positive thigmotaxis, and partly because contact stimuli (as shown in a previous paper) cause it to become negatively phototactic. The positive phototaxis which appears when the insect is swimming freely in the water is certainly of no service to it in leading it into its accustomed habitat if it should, for any reason, become removed from it. In the air and near a bright light *Ranatra* becomes, sooner or later, strongly positive, often being wrought up to the highest pitch of excitement in its efforts to reach the light. Of the utility of such curious behavior it is indeed difficult to conceive a reasonable explanation.

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MADISON, WIS.

A STUDY OF FUNDAMENTAL BARS IN FEATHERS.

OSCAR RIDDLE.

The structure and development of feathers have been studied by many investigators. The pigments of feathers have also been the subject of a very great number of researches. In spite, however, of these numerous studies of feather structures and pigments, we know almost nothing of structural differences between pigmented and non-pigmented areas, and nothing at all of the causes which lead to the orderly and definite distribution of pigment into the often complex color-patterns commonly found in birds. In connection with a research directed to these two points it was thought advisable to make a study of certain *defects* which were known to appear occasionally in feathers. It is a preliminary account of my results in this more restricted field — which, however, proved to be a rather significant one — that is to be found in this paper. The work was undertaken in 1904 under the direction of Professor C. O. Whitman in the zoölogical laboratory of the University of Chicago. It is a pleasure to acknowledge the help, encouragement and criticism which Professor Whitman has given in connection with this work.

At the time I took up the study of the defects under consideration, they had been reported but once, and this report had to do with but a single specimen, a single plumage, and a single defect in each feather. This was an account by R. M. Strong[1] of "A Case of Abnormal Plumage" found in a hybrid pigeon. Dr. Strong described and figured two types of abnormalities, and concurred with Professor Whitman, who had reared the bird, in the opinion that the defects were probably caused by malnutrition during the growth of the juvenal plumage. Besides this case, Professor Whitman had observed these or similar defects in the feathers of several of his birds, and my problem was to learn the extent of their occurrence and to determine their cause. In the course of my studies I have found still other abnormalities —

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[1] BIOLOGICAL BULLETIN, November, 1902.

or rather, different forms of the same defect — and I shall first give a description of the nature of the defects, and later consider the question of their extent and cause.

Defects in Adult Morphology. — In the adult expanded feather, I have found five types of defects in structure. In Fig. 1, *a* is shown the first type. There is in this case a sharply defined area extending entirely across the feather-vane, in which there are no, or very few, perfect barbules. A cross-section of the feather at this point would show only shaft and barbs. One such area in the entire length of the feather was one of the types described by Strong. I find, however, an abundance of cases where such areas occur at regular intervals practically throughout the length of the feather. This regularity in the spaces separating the defects, indeed, furnished the clue to the nature of the latter. It will be seen that these areas cross the barbs in such a way as to form almost a right angle with them. The same thing is true of the other types of defects and argues for their standing primarily all for the same thing.

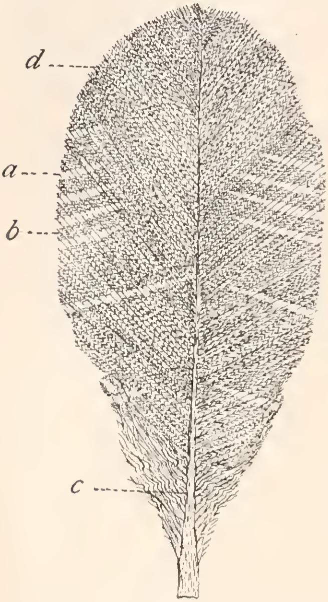

Fig. 1. Feather from a poorly nourished chick showing abnormalities. *a*, abnormal area; *b*, 'fundamental bar' (a day's growth); *c*, constrictions; *d*, region in which defective lines showed plainly in this feather. (× 2.)

The second type represents the greatest extreme to be met with among these abnormalities. The feather in the abnormal region has been reduced to shaft only; both barbules and barbs are gone. The second of the defects described by Strong evidently belonged to this type, though he states that there was no shaft present in his material and that its place was taken by a small cylinder of fused barbs. I have not seen just such a structure as he describes; but it is rare and doubtless is to be regarded as a sort of record of the very severest conditions which a bird can encounter and endure. In types one and two the barbs and shaft are often bent or kinked in the abnormal region.

The third type of defect is something very much less conspicuous than either of the two types already considered. It could not be represented in the drawing. It is a very minute depression extending across the upper and dorsal surface of the feather. It is not always easy, however, to determine that it is a depression at all. It often seems a line, or simply the point of union of a distal with a proximal part of the feather-vane. This line crosses a series of barbs making with them a right angle as did the defective area of type one. These lines or depressions are usually so inconspicuous that even close observation may not reveal them. Yet they exist and can be demonstrated in all feathers, and at any level throughout the length of the feather.

The existence of these depressions as normal occurrences in the feather is apparently nowhere mentioned in the literature. Certainly their significance has not been made known. As may be inferred from my classification of them, I have found them to bear a close relation to the defective feather areas. These lines which are thoroughly characteristic of feathers are properly classified among feather defects, for, it is always at these lines that the defects like those of types one and four appear and, moreover, they show all possible gradations into types one and four. I shall hereafter speak of defects of this type as *defective lines*, or depressions; those of type one as *defective areas*; those of type four as *constrictions*.

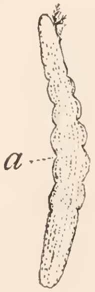

FIG. 2. Entire feather-germ from *Cardinalis virginianus* showing constrictions. (Actual length 13 mm.)

It is those feather-vanes which are made up of a series of deep depressions or constrictions that show the defects of type four. I shall say nothing here of the conditions represented in this type, but perhaps an idea can be had from the feather germ shown in Fig. 2.

Of type five, I have seen but a single example. In this the defect extends vertically or the long way of the feather. The barbs of one half of the vane have their distal portions broken away at even distances from the shaft. Duerden[1] states that this abnormality is also very rarely met with among ostriches.

The recognition of the defective lines in all feathers throws a new light on abrasion and wear in feathers. That there are birds which "normally" have the barbules broken off at certain fairly definite points in the more distal barbs has been observed by Meves,[2] Chapman,[3] Dwight,[4] Strong[5] and others. Meves and Chapman have noted, too, that the barb itself may be broken near the distal end. I have seen several cases of the breaking of a series of barbs at the point where they were crossed by the same defective line, and I believe that further study will prove that most feather abrasions occur by the space between two defective lines breaking away as a single piece.

The Defects in Feather-germs. — I have been able to observe the defects in several formative stages. I shall say only a word concerning them here. The prominent defects in the unexpanded germ are easily recognized by the unaided eye; sometimes they appear as definite constrictions (*Cardinalis virginianus*, see Fig. 2, *a.*), but very often as points of a different color (rectrices of *Turtur risorius*). A microscopic examination of a region which would develop a defective area shows a reduction of cell-growth and division particularly in the region of the barbules.

EXTENT AND DISTRIBUTION ON THE ABNORMALITIES.

In the Bird Groups. — In looking for the cause of the defects one turned naturally to the birds to find whether they were widespread or restricted phenomena. I stated that at the time the present work was begun, there was in the literature but a single account of them, and that account had to do with a single specimen — a hybrid pigeon. Recently Professor J. E. Duerden has reported the abnormality in the ostriches, particularly in those of South Africa. I learn from him by letter that he has undertaken a thoroughgoing research to determine the cause of the "barring" so prevalent in the ostriches. He estimates that the value of the ostrich plumes from South Africa alone are from this cause depreciated in value to the extent of £250,000 annually.

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[1] Duerden, "Bars in Ostrich Feathers," *Agr. Jour. Cape of Good Hope*, May 1906.

[2] Meves, W., "Über die Farbenveranderung der Vogel," *Jour. für Ornith.*, Bd. 3, 1855.

[3] Chapman, F. M., "On the Changes of Plumage in the Snowflake," *Amer. Mus. Nat. Hist.*, vol. 8.

[4] Dwight, J., Jr., "The Sequence of Plumages and Moults in the Passerine Birds of New York," *Ann. N. Y. Acad. Sci.*, vol. 13, No. 1.

[5] Strong, R. M., "The Development of Color in the Definitive Feather," *Bull. Mus. Comp. Zool.*, vol. 40, no. 3.

The defects are, however, not confined to hybrid pigeons and domesticated ostriches. I find them in the most widely separated bird groups; in primitive and in recent birds; in land and in water birds; in domesticated and in wild birds; in birds from the arctic and from the torrid zone, etc. I have been able, owing to the courtesies extended by Professor C. B. Cory and Dr. Ned Dearborn, of the Field Columbian Museum in Chicago, to examine a very great variety of birds belonging to the Museum. I find that although it is not easy to see evident defects in every specimen, it is easy to find them in every species. We may conclude therefore, that they are to be found in *all* birds.

It is a fact, and a significant one I think, that the defects are, in general, more common in domesticated and caged birds than in wild birds. In this connection, however, it should be stated that the defects appear indifferently in pure breeds, hybrids and mongrels. At any rate I have verified this in a number of our domesticated birds.

On Individual Birds. — I have found the defects in all of the plumages of the birds, with the possible exception of the first or downy plumage. In some birds the defects seem to occur more frequently in the juvenal (of Dwight) than in the others. The emphasized defects appear in all the feather-tracts or pterylæ; but in a particular bird, and usually in a particular species, certain tracts show them in greater numbers than do others.

In an Individual Feather. — In the feather there may be produced at any point in its length, either of the five types of abnormality. In some birds (*Gallus*) the distal part of the feather oftener shows the defective *areas;* the proximal end, the deep *constrictions*, while we get defective *lines* in one form or another at every point in the feather's length.

The Meaning and Cause of the Defective Lines and of the Spaces Between Them.

We may now consider the significance of this blocking out of the feather from end to end into bands, "bars," or plane feather-elements, separated from each other by extremely faint depressions or constrictions — for, my studies demonstrate that this is a true conception of feather structure.

That the feather from tip to tip does not represent a perfect, uniform continuity, but is made up of an apposed series of faint "fundamental bars" is a conception which I owe to Professor Whitman. I have proved absolutely that the defective lines, or points of apposition of the "fundamental bars" are the points at which all of the defects appear, and are therefore, really miniature representatives of the defective areas and constrictions of types one and four. I think I have also proved that each block, segment or "fundamental bar" of the feather represents a day of growth, and this is at the same time the amount of feather-growth between two low blood-pressures. Further, I have abundant evidence that the defective lines and areas represent points developed under a diminished rate of cell-growth and cell-division, brought about by a reduced nutrition, which is in turn the result of a daily lowering of the blood-pressure. This low blood-pressure doubtless occurs between one o'clock A. M. and six A. M.

The evidence that a single "fundamental bar" and a single defective line or area are laid down each day, and that this is the total of a day's growth is conclusive. In very favorable material I have been able to show, for example, that a feather 56 days old shows 56 "fundamental bars" and 56 defective lines, areas and constrictions. That the defective area is laid down at night and during a period of low blood-pressure, I have demonstrated twice experimentally. A chick was kept on two succeeding nights, from 8 o'clock P. M. till 8 A. M. in an atmosphere containing amyl nitrite (which lowers the blood-pressure).[1] This bird later showed two emphasized defective areas in the region of the feather produced during the two days of the experiment, and these areas occupied the region normal to the defective lines and did not appear in the territory occupied by a " fundamental bar." Since these defective lines are laid down at approximately the same time each day — as is proved by the regularity in the distances separating them — we are forced to the conclusion that the defective *lines* are normally laid down at night, and that a lowering of the blood-pressure is associated with the production of defective *areas*, and, therefore of defective lines, for, that the defective line stands for the initial stage of the defective area is as certain as that an area has more dimensions than a line. The evidence in part is, that one sees all possible intergradations, that each marks off a day's growth, that when the area occurs it always falls in the place for the line, that a certain part of the line only may be transformed into the obviously defective area, etc. That there is a reduction of cell-growth and cell-division in the defective area is proved absolutely by an examination of the adult morphology of an exaggerated defect, as it is also by the histology of the defects in the feather-germ.

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[1] The effect of several drugs on the blood-pressure of birds has been investigated by Dr. S. A. Matthews and the writer. Our results are soon to be published.

That the low blood-pressure occurs at night is evidenced by the experiment of the chick in the amyl nitrite. That it occurs between midnight and six in the morning may be inferred from the fact that the lowest daily *temperature* in birds falls between these hours. Reasoning from the facts known in mammals we may assume that the minimum blood-pressure coincides in point of time with the minimum temperature. I have not been able to get the daily blood-pressure curve of birds, owing to the difficulty of doing so in birds of small size. The ostriches might well be used for that purpose. At this point I may suggest that the ostriches will doubtless cease to interpolate defective *areas* in their plumes as soon as they can find the perfect diet, and the various life conditions which will give them *well-nourished bodies* and strong, *effective circulations.* After all, these two are one.

The Relation of Nutrition to the Defects.

At the very beginning of this study, it was thought that the defective areas stood in a certain relation to a faulty nutrition. A number of experiments were made to determine this. A number of young ring doves were alternately starved and fed, with the result that in these birds the defective areas appeared in the juvenal plumage in great numbers. The same experiment was tried on young chicks with the same result. It was noticed, however, that notwithstanding the careful and plentiful feeding of the control, an occasional defect could be found in their feathers too. These experiments[1] showed that malnutrition is beyond doubt the important factor in the production of the defective areas, but apparently not the only one. An experiment was then carried through to learn whether the defects found in the control could be produced by the usual *handling* of the birds, and perhaps slightly crumpling their feather-germs. The results were negative; but it was found that when the feather-germs were *strongly crumpled* and broken in the region of feather growth, the defects were readily produced.

During the progress of an experiment on some young chicks (carried on for a quite different purpose) it was found that chicks which were fed on the fat stain Sudan III produced the defective areas in much greater numbers than did the control birds. It was determined that the ordinary variations of light, temperature, etc., did not cause the defects. The net result so far of all the experiments for the determination of the cause of the defects, indicated that those things which interfere with the *nutrition* of the feather-germ will produce the defects, while those things not capable of affecting the nutrition will not produce them. It is easy to understand how a crumpling of the feather-germ would temporarily interfere with the circulation within it. In the chicks fed with Sudan III. it was evident that a sort of "starving" effect was produced by it. By the time the experiments had proceeded thus far I knew that a day of normal growth in a feather is represented by a "fundamental bar" and a defective line, and also that a defective line stands in close relation to a defective area. This suggested that the defective areas in the control, and the defective lines in all feathers, are produced by an internal factor with a definite rhythm, and that the rhythm is able, like my experiments, to effect the nutrition of the feather elements. This recommended blood-pressure to me, and the experiments were made with the result stated above.

Blood-Pressure and Temperature Rhythms. — I shall not here attempt to explain the causes for the nightly fall of the blood-pressure in birds. Let it suffice to say that we find a parallel phenomenon in mammals. I wish further to call attention to the fact that my demonstration that the lowest blood-pressure in birds falls at night is evidence that the blood-pressure and temperature curves of birds are similar curves, as they are known to be in mammals. Of course, I have not showed a blood-pressure *curve* for birds; I have, however, located in a general way the *time* of its *minimum*. I am in a position to confirm the observations of Corin and Van Beneden[1] as to the temperature curves of birds. They worked with pigeons. I have temperature curves essentially similar to theirs, from ducks, ring-doves, and from chicks both old and young. The lowest temperature occurs at about four to five o'clock A. M.

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[1] Partial results of these feeding experiments were communicated by letter to Professor Duerden and were published in his paper, cited elsewhere.

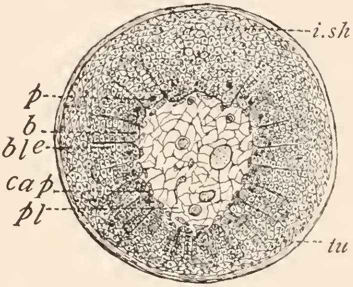

FIG. 3. Cross-section of a feather-germ in the region of growth. (Semi-diagrammatic, magnified about 100 diameters.) *b*, barb-forming cells; *ble*, barbule-forming cells; *p*, pigment cell; *cap*, capillaries; *pl*, pulp; *tu*, outer sheath; *i.sh*, inner sheath.

Low Blood-Pressure and the Nutrition of the Feather Elements.— There remains to be indicated some of the histological relations of the capillaries and the feather-elements which suffer from the lowering of the vascular tension. I shall also outline the way in which the low pressure probably acts.

Just as among the vertebrates we know that certain tissues, *e. g.*, the liver cells, are kept always on the verge of asphyxiation, so I believe are the epidermal cells of the growing feather-germ taxed to their utmost to secure from the blood enough nourishment to allow the rapid cell-division to proceed in full swing. Where else in an adult vertebrate do we find a more rapid growth and differentiation of tissue than we find in the moulting of certain birds? We may then expect to find here a struggle for food when this becomes reduced in amount, and those parts nearer the blood-supply should fare better than parts

[1] Corin, G., and Van Beneden, A., "La Régulation de la températrure chez les Pigeons," *Archives de Biologie*, Vol. VII., pp. 265–276, 1887.

more removed. Now this is exactly what happens. The capillaries (Fig. 3 *cap*) of the feather-germ lie nearest those cells which enter into the formation of the *barbs* (Fig. 3, *b*) and these are able to continue to grow even with a weakened food-supply; they too, though, are suppressed in cases of extreme starvation. The cells which form the *barbules* (Fig. 3, *b*) are not in contact with capillary walls, and can utilize only the surplus of food which filters through the barb-forming cells. With this fact in mind it is clear that we should expect a diminished food-supply to first check the growth in the barbules and that still further reduction is necessary to check the growth of the barbs. Experience proves that this is true (I use the words "food" and "nutriment" in a broad sense, and *oxygen* is to be read into them). It is conceivable that a reduced oxygen-supply is here playing a part, since in all my experiments and in any normal lowering of the blood-pressure, the available oxygen is decreased.

From what has just been said of the filtration method by which the barbule cells receive their nutriment, we can now see how it is that blood-pressure plays so important a part in the production of defective areas. It is well known that when a period of low blood-pressure sets in, the lymph begins to flow from the spaces between the cells of the body into the capillaries. Thus, by withdrawing a quantity of food from the immediate environs of the cell, a low blood-pressure affects the cell in the same way as does an actual reduction of the amount of nourishment circulating in the blood.

It appears, therefore, that the feather germ as it grows and unfolds, spreads before us a record of some earlier significant occurrences within. Indeed, it now seems certain that the delicate filaments, so admirably interlaced to form a feather-vane, are as capable as a revolving drum of recording the important changes in vascular pressure. To be sure, the tracings on the plumes are not to be measured as symmetrical curves with a definite number of millimeters of daily variation, but are written in "fundamental bars" separated by areas more or less imperfect which are to be read in terms of cell-growth and cell-division.

Hull Zoölogical Laboratory,
University of Chicago,
December, 1906.

STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

II. Development of the Testes and Spermatogenesis in Moniezia.

C. M. CHILD.

The material for this study was obtained from the same species, *viz.*, *Moniezia expansa* and *Moniezia planissima*, as that of the first paper of this series[1] and most of it, in fact from the same chains. The methods of fixation, observation and record are the same as those already described in that paper. Various details in the spermatogenesis are only briefly considered, since they are not directly connected with the chief purpose of the paper.

I. *The Formation of the Testes.*

The testes develop from cells of the parenchyma which do not differ visibly from other cells of the same region. They appear in the dorsal region of the central parenchyma. Before their appearance two kinds of cells are visible in the parenchyma: one of these is smaller and surrounded by more or less cytoplasm apparently without definite boundary and usually elongated in the dorso-ventral direction, with one or more fibrillar extensions at each end. Figs. 1, *A–D* (Pl. VII.), show parenchymal cells of this kind in the earliest stages of testis formation. Amitotic division of the nucleus is occurring in each case. Fig. 1, *A*, shows a case in which the two parts of the dividing nucleus stain differently Fig. 1, *C*, a case of the endogenous form of nuclear division which was described in the preceding paper, while Figs. 1, *B*, and 1, *D*, show late stages in division, the one by constriction, the other by formation of a nuclear plate. For a description of these and other forms and stages of amitosis in *Moniezia* the reader is referred to the preceding paper of this series.

The other form of cell existing in the parenchyma before testis formation is much larger, and its cytoplasm is highly vacuolated and more distinctly bounded from the parenchymal matrix about it. These cells appear always to be connected with deeply staining fibers which extend dorso-ventrally across the central region of the proglottid and which I take to be dorso-ventral muscle fibers since they and the cells connected with them are similar to those of the muscular layers. Fig. 2, *A* (Pl. VII.), shows one of these cells with a portion of its fiber. The fiber passes directly through the cell-body on one side of the nucleus and the cytoplasm extends visibly for a considerable distance along the fiber. The evidence that these cells develop into testes is very strong. Fig. 2, *B* (Pl. VII.), represents a section through nucleus and body of one of these cells in which the nucleus is apparently undergoing amitosis. Whether this particular case would have developed into a testis it is of course impossible to determine. But Figs. 2, *C*, and 2, *D* (Pl. VII.), represent characteristic cases slightly more advanced. Here several nuclei are contained in a space which corresponds closely with the form of the muscle cell and contains what seem to be strands of the old vacuolated cytoplasm, while about some of the nuclei a layer of more deeply staining cytoplasm is visible, apparently in process of formation. Through the space which apparently represents the region previously occupied by the body of the muscle cell passes the fiber. The presence of the fiber and the well marked outline of the space seem to me to constitute very strong evidence in favor of the conclusion that each of these groups of nuclei have arisen by the division of a nucleus of a muscle cell. That these groups develop into testes there can be no doubt. Their development can be followed from proglottid to proglottid without the slightest difficulty, and there are no other similar groups of nuclei in the parenchyma. Fig. 2, *E* (Pl. VII.), shows a case in which the muscle fiber is apparently undergoing degeneration. In all of these cases amitotic division of the nuclei is taking place. The figures give only a few examples of the cases observed. In a brief account of the history of these cells already published additional figures are given.[1] If these observations are correct, and I have, so far as

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[1] Child, "Studies on the Relation between Amitosis and Mitosis I. Development of the Ovary and Oögenesis in Moniezia," Biol. Bull., Vol. XII., No. 2, 1907.

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[1] Child, "The Development of Germ Cells from Differentiated Somatic Cells in Moniezia." *Anat. Anz.*, Bd. XXIX., Nos. 21 and 22, 1906.

I am aware, taken all possible precautions to assure myself that they are, it seems impossible to escape the conclusion that in *Moniezia* the male germ-cells may develop from cells which have previously been differentiated and functional in the soma. Generalization from this conclusion is, however, no more justifiable than is that so often made from observations which seem to point in the opposite direction. It does not seem probable that uniformity exists here any more than in other features of development in regard to which premature generalizations mark like wrecks the dangers along the channel of biological thought.

In slightly later stages it is difficult or impossible to determine with certainty which testes have arisen from the smaller and which from the larger cells. Figs. 3, *A*–3, *F* (Pl. VII.), represent young testes which probably developed from the smaller parenchymal nuclei: amitosis is visible in all cases except Fig. 3, *F*, in which mitosis is occurring. In the hundreds of testes examined at this stage four cases of mitotic division have been observed of which one is shown in this figure. All of the observed cases were found in a single chain of *Moniezia expansa*, another fact which seems to indicate that the relative frequency of the two forms of division may vary. It was not possible to determine the number of chromosomes with certainty, but it was more than twelve, the number shown in the figure.

Figs. 4, *A*–4, *D* (Pl. VIII.), represent cases which probably developed from the large cells — muscle cells. Such stages are found in the same proglottids as the stages shown in Figs. 3, *A–F*, and are clearly larger and contain more nuclei than the latter. Fig. 4, *A*, shows a case in which a small nucleus, apparently not a part of the large mass, is also seemingly involved in the development. Cases of this sort are not infrequent, and the small nucleus often becomes one of the membrane-nuclei, though the latter appear in many cases to arise from the same primordium as the germ-cells themselves. Here, as in the development of the female organs, it is difficult to resist the impression that the development of these organs is the result of some localized stimulus or condition and that any cells within reach of this factor may become involved.

At this stage the nuclei of the developing testis lie in a continuous mass of cytoplasm, which is more or less distinctly marked off from the parenchymal substance, but still shows fibrous extensions into the parenchyma. The development of the testes differs from that of the ovary in that none of the parenchymal substance is included within the testis. This difference is merely the consequence of the fact that the testes usually develop from a single nucleus and its surrounding cytoplasm, while the ovary develops from a large number of the parenchymal nuclei.

II. *The Growth of the Testes Preceding the Spireme Stage.*

During the period preceding the appearance of the spireme in a part of the cells the development of the testes consists of increase in size as the result of numerous divisions, chiefly amitotic and of formation of the membrane about the testis and of the vasa efferentia.

The formation of the membrane occurs at an early stage from the cytoplasm of cells about the periphery of the proliferating mass. Fig. 5, *A* (Pl. VIII.), shows one of these cells with the membrane forming an extension of the cytoplasm. The vas efferens is formed in the same manner (Fig. 6, *A*, Pl. VIII.). The membrane-forming cells are few in number and the nuclei apparently undergo degeneration in later stages, for they are very rarely found in the fully developed testis.

The contents differ somewhat in appearance according to the method of fixation employed. After Hermann or chrom-oxalic the nuclei appear in most cases to be imbedded in a syncytial mass of cytoplasm, cell boundaries being indistinguishable or sometimes faintly visible, though cavities or vacuoles are frequently observed. After sublimate and some of the sublimate mixtures in all but the earlier stages the cytoplasm appears to have undergone shrinkage and to be more or less definitely concentrated about each of the nuclei. In later stages the individual cells appear more distinct after any fluids. The earliest stages of testis-development are certainly syncytial and without doubt the individualization of the cells takes place gradually. In consequence of the shrinkage of the delicate and probably highly fluid protoplasm caused by the sublimate fluids the distinctness of the cells is exaggerated. Most of the figures of these stages (Figs. 5, *A*–6, *B*, Pl. VIII., 7, *A*–8, *B*, Pl. IX.) are taken from the chrom-oxalic preparations and cell-boundaries do not appear in most cases though they may be present. Fig. 9, *D* (Pl. IX.) is taken from a sublimate preparation at a rather late prespireme stage and shows the cells as distinct.

But for present purposes the nuclei are of chief importance. Figs. 5, *A*–5, *C* (Pl. VIII.) represent stages just after the formation of the membrane. In Figs. 5, *A*, and 5, *B*, amitoses are visible and in Figs. 5, *B*, and 5, *C*, two of the very infrequent cases of mitosis in these stages are shown. Figs. 6, *A*, and 6, *B* (Pl. VIII.) are from slightly later stages: Fig. 6, *A*, is a section through one side of a testis and does not show the full size. Both figures show amitoses and Fig. 6, *B*, shows one case of mitosis. Figs. 7, *A*, and 7, *B* (Pl. IX.), are from still later stages. In the latter figure one case of division of a nucleus into three parts is shown. The nuclei in which such divisions take place are usually larger than the others, often lie near the center of the testes and are similar in appearance to the large nuclei along the axis of the developing ovarian follicles which were mentioned in the preceding paper. In Figs. 8, *A*, and 8, *B* (Pl. IX.), stages just preceding the first appearance of the spireme, each with several amitoses, are shown. In Fig. 8, *A*, one of the large nuclei dividing into three parts is seen near the opening of the vas efferens.

In Fig. 9, *A*–9, *D* (Pl. IX.), cells or cell-groups from various stages containing amitoses of special interest are shown. Figs. 9, *A*, and 9, *C*, represent cases of the form of amitosis designated endogenous in the preceding paper in which the two nuclei resulting from division do not occupy the entire space within the old membrane. Numerous cases of this sort have been observed in the testes and have been examined with great care. Fig. 9, *B*, is a case of triple division and Fig. 9, *D*, a case of cytoplasmic division proceeding from within outward. This figure is from a sublimate preparation.

During these stages mitoses are rare. Very often not a single case is found in any of the numerous testes of a proglottid. In other proglottids a number of testes may show one or more each. In general the relative frequency of mitosis appears to vary in different chains and in different proglottids. In one chain of *M. planissima* for example mitosis has been observed only very rarely though the chain has been carefully examined; in another it was found to be much more frequent. In all cases, however, amitosis is the predominant form of division in these stages.

III. *Formation of the Spireme and the Growth Period.*

In the stages before spireme-formation, or as it has often been called, synapsis, all the nuclei in the testes are similar in appearance and contain a large deeply staining nucleolus with perhaps a few smaller granules.

Suddenly a part of the nuclei begin to increase in size and a spireme appears (Figs. 10, *A*–13, *B*, Pl. X.). The formation of the spireme takes place in the manner described for the ovary in the preceding paper of this series. The change does not appear to begin in any particular region of the testis. Sometimes different groups of cells in different regions of the same testis give rise to a spireme while about them and between them lie others still unchanged and undergoing amitosis. From the first appearance of the spireme in the testes until the formation of spermatozoa is completed the multiplication of the spermatogonia which remain in the prespireme stage goes on, chiefly or wholly by amitosis and some of the cells thus produced are continually passing into the spireme stage. Consequently the stage is not characteristic of any particular period of development of the testis as a whole after its first appearance; in the older testes some groups of cells in the spireme stage and some groups of spermatogonia in prespireme stages are always to be found.

In some testes before the spireme stage appears and frequently afterward some of the cells are seen to be more or less pear-shaped in form with the pointed ends radially arranged about a center and united by strands of cytoplasm (Figs. 10, *A*, 11, *B*, Pl. X.). The number of cells in a group of this kind varies from three or four to eight or ten. All the cells of a group pass into the spireme stage simultaneously. Whether such groups are due to the persistence of cytoplasmic connections from previous divisions or to the formation of new connections it has been impossible to determine, but it seems possible from the varying size of the groups that they are merely the result of connection of cells lying near each other.

The grouping of the cells is not by any means a characteristic feature in these stages. Frequently cells which are entirely separate from each other pass into the spireme stage simultaneously (Figs. 10, *B*, 11, *A*, Pl. X.). As will appear below, the grouping is merely the first step in a process characteristic of spermatogenesis and observation indicates that in some cells it begins before the spireme appears in others not until later.

As in the ovary the spireme is usually massed at one side of the nucleus as in many other cases of synapsis and is often visibly connected with the nucleolus (Figs. 12, *A*–12 *B*, Pl. X.), which, however, does not decrease in size but increases as the nucleus grows larger (Figs. 10, *A*–13, *B*, Pl. X.).

The appearance of the spireme is accompanied by an increase in the amount of cytoplasm. Comparison of the cells in this stage with those in earlier stages in Figs. 10, *A*, 10, *B*, and 11, A (Pl. X.), shows this difference clearly. The cytoplasm in the spireme stages also appears somewhat more dense in structure and stains a little more deeply. Here as in the ovary the apparent connection of the nuclear changes with the growth of the cytoplasm is most striking.

As is usual, this stage in the testes is not accompanied by such extreme growth of the cytoplasm as in the ovary nor by any formation of yolk, but is soon followed by the spermatogenetic divisions.

But the stages following the spireme are not the same in all cells ; the later development follows two very different lines. In the later stages of the spireme period it is possible to distinguish two different sorts of nuclei. In the one (Fig. 13, *A*, Pl. X.) the nucleolus has disappeared and the spireme is very dense and occupies almost the whole periphery of the nucleus. Careful examination and comparison has convinced me that these nuclei are in preparation for the first spermatocytic mitosis. The nuclei of the other sort are considerably larger (Fig. 13, *B*, Pl. X.), the spireme is much less dense and more irregular in form and does not occupy the whole periphery but is still massed more or less at one side and the nucleolus is still intact. I am confident that these nuclei do not represent earlier stages than those in Fig. 13, *A*, for they are always larger than the latter and the irregularity of the spireme is not found in the earlier stages. These nuclei are the first stages in a remarkable process of fragmentation which will be described in a later section. Their relative frequency as compared with the others appears to vary in different chains, proglottids and regions. Sometimes they seem to be more, sometimes less numerous than the others.

(To be continued.)

EXPLANATION OF PLATES.

PLATE VII.

FIG. 1. *A–D*, testes forming from parenchymal cells: amitotic division in all cases.

FIG. 2. *A*, muscle cell; *B*, muscle cell undergoing amitosis; *C*, *D*, *E*, muscle cells developing into testes; in *E* the muscle fiber is apparently undergoing degeneration.

FIG. 3. *A–F*, young testes; in *F* a case of mitosis.

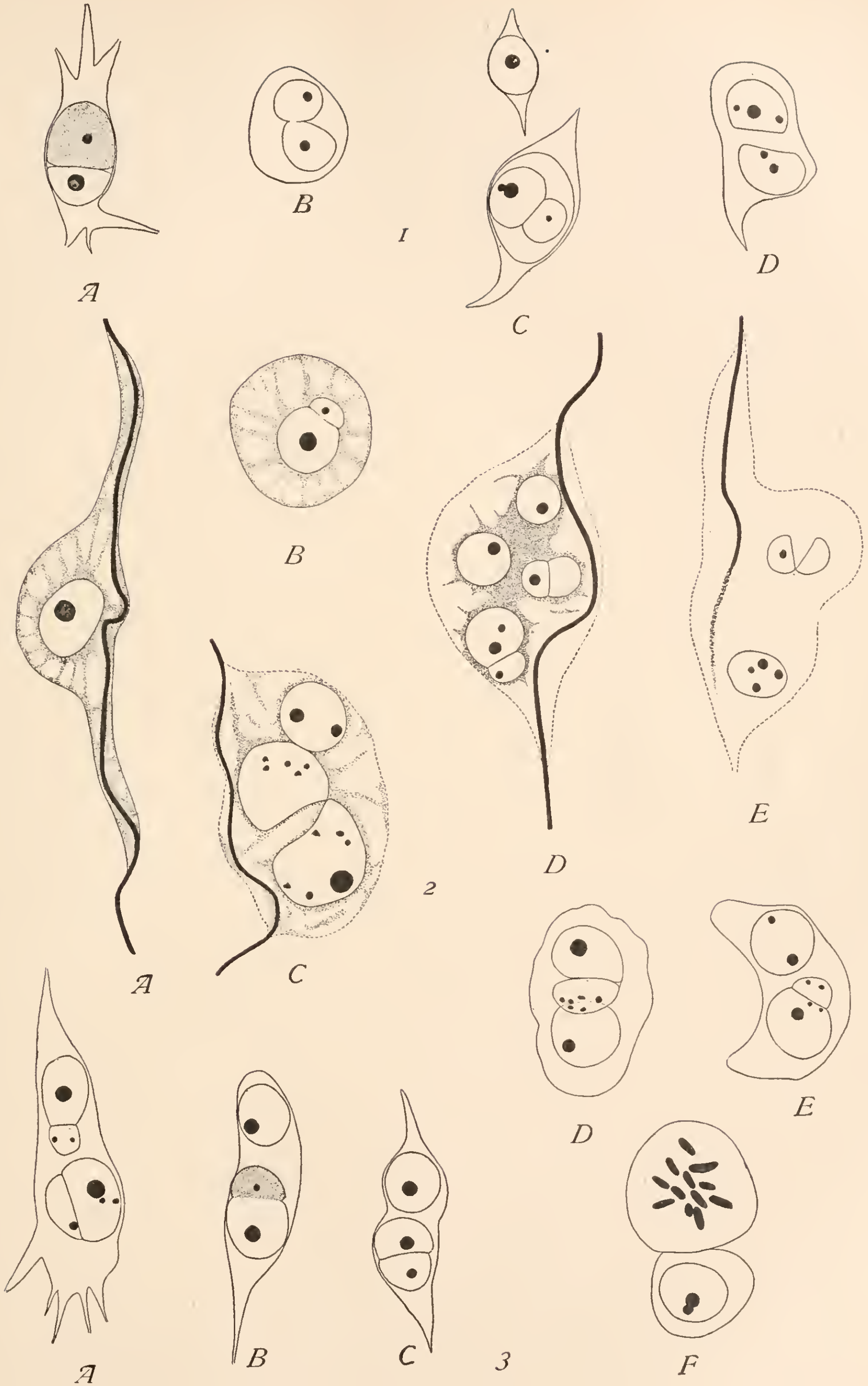
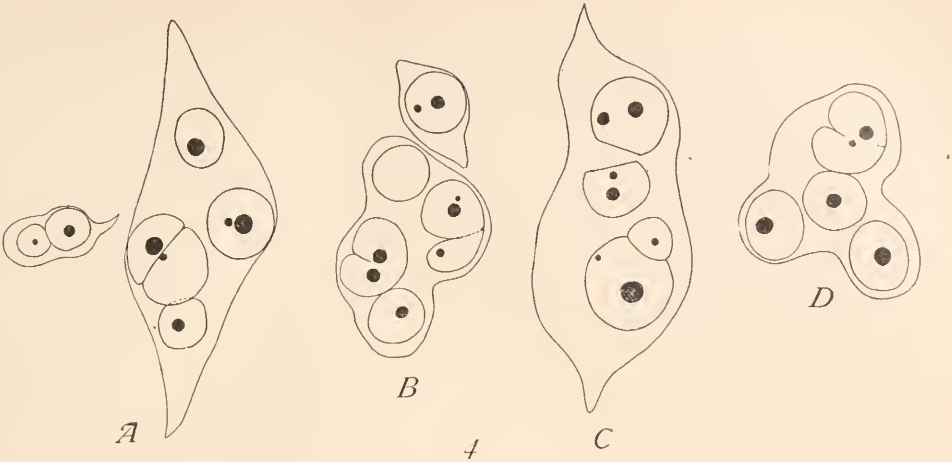

A
B
1
C
D
A
B
C
D
E
2
D
E
A
B
C
3
F

PLATE VIII.

FIG. 4. *A–D*, young testes showing amitosis.

FIG. 5. *A–C*, young testes after formation of membrane and vas efferens; *B* and *C* contain mitoses.

FIG. 6. *A*, *B*, developing testes.



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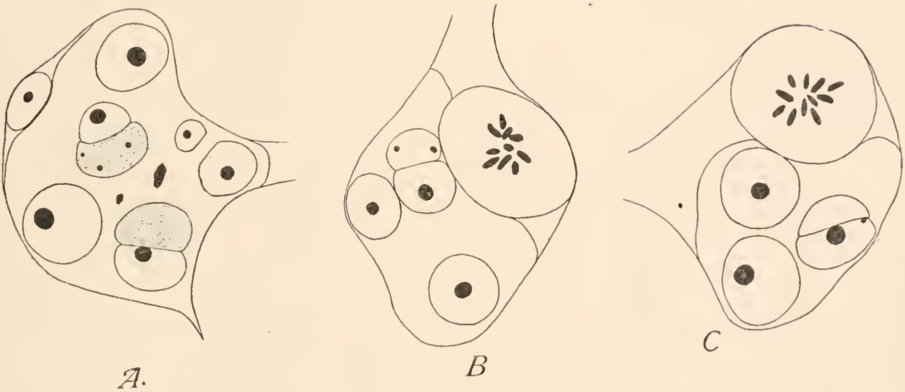

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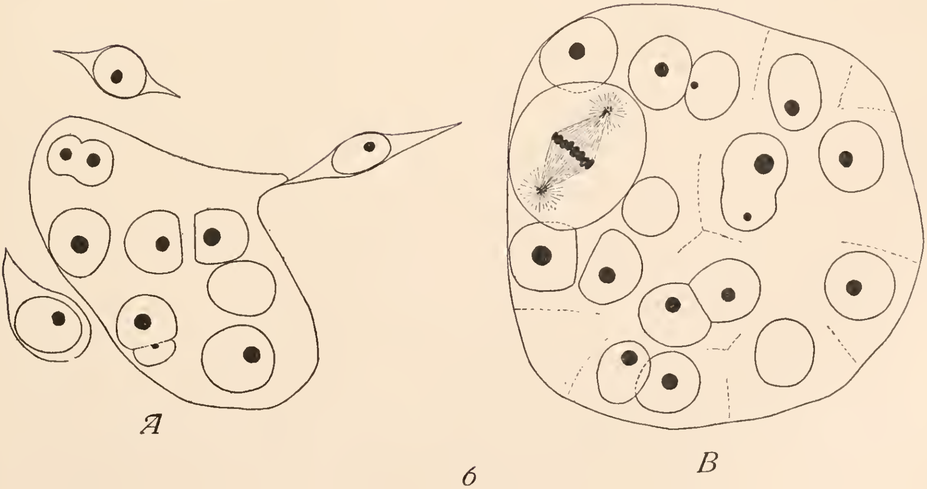

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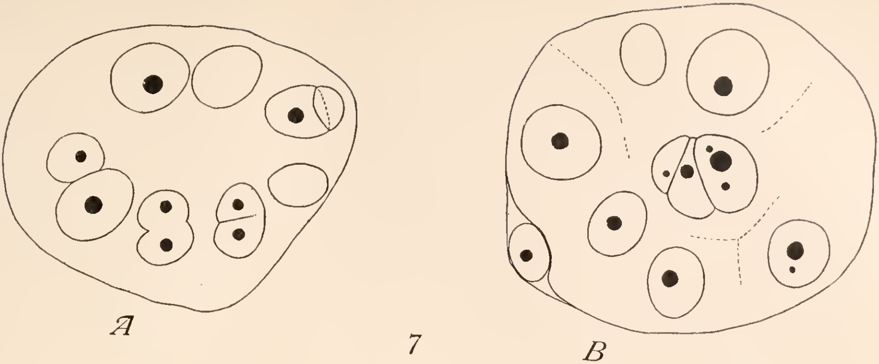
PLATE IX.

FIG. 7. *A*, *B*, developing testes.
FIG. 8. *A*, *B*, developing testes in later stages.
FIG. 9. *A–D*, amitoses from developing testes.



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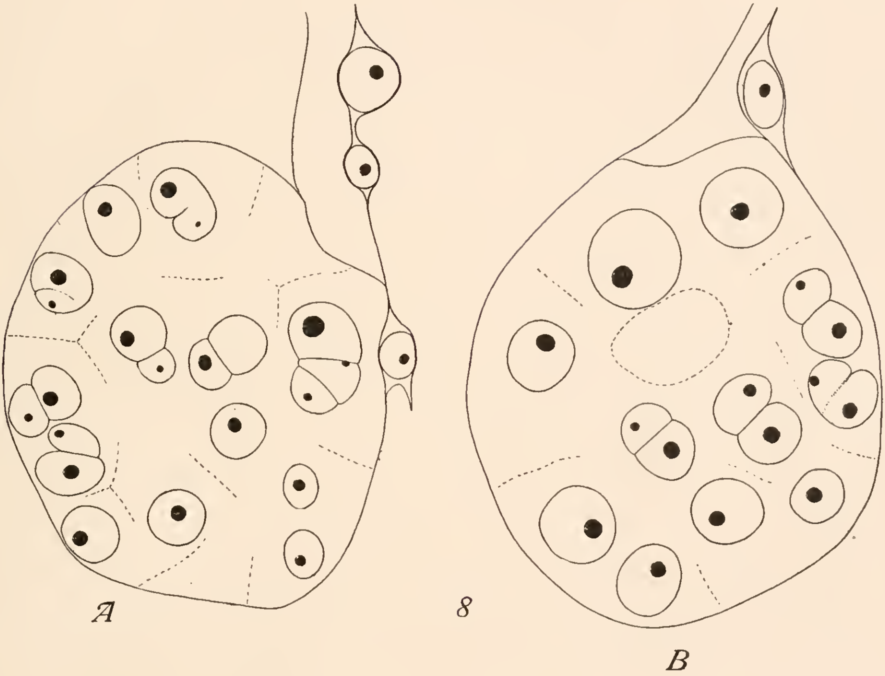

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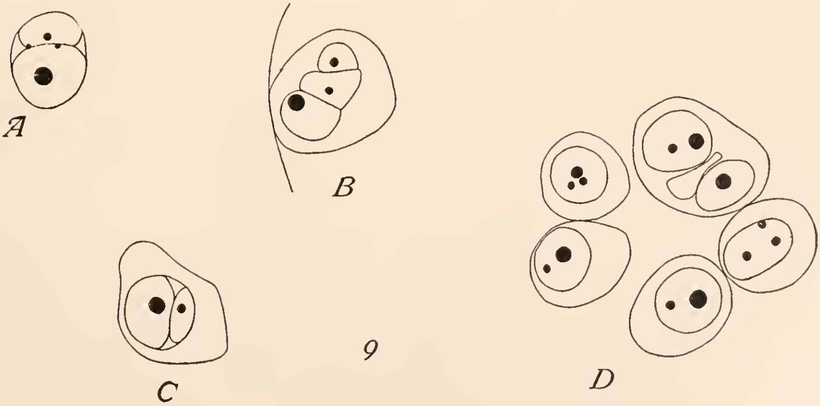

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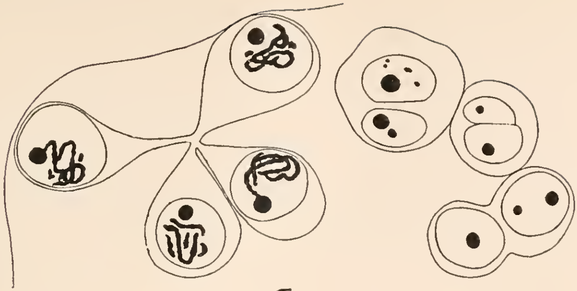
Plate X.

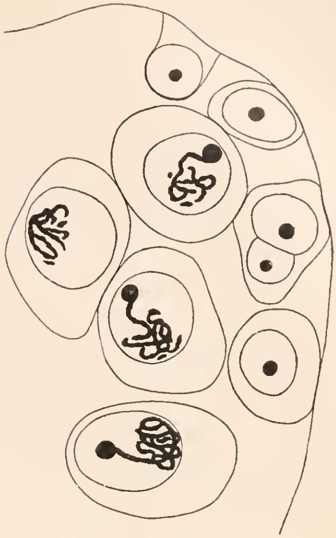
Fig. 10. *A*, *B*, Fig. 11; *A*, *B*, the spireme stage. Figs. 10, *A*, and 11, B, show the first stages in fusion of the spermatocytes to form a cytophore.

Fig. 12. *A*, *B*, spireme stages.

Fig. 13. *A*, preparation for first spermatocytic mitosis; *B*, preparation for fragmentation.

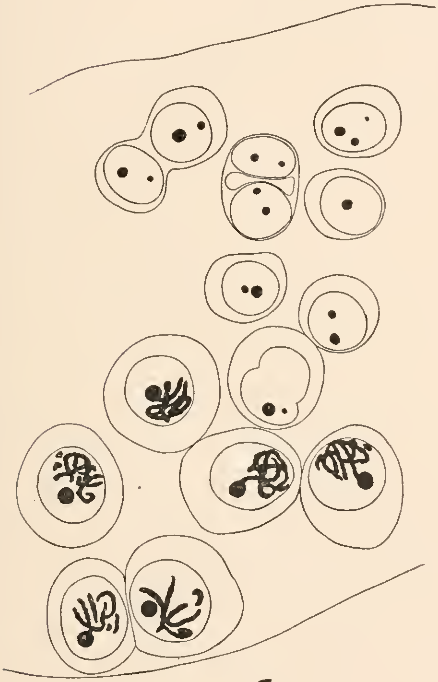


A



B

10



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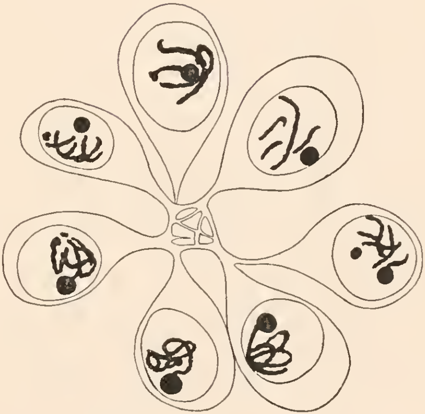
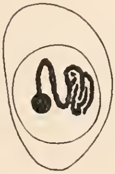
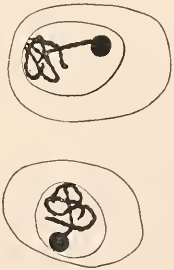


B

11

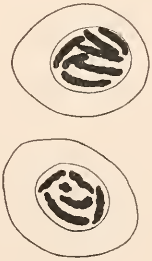


A

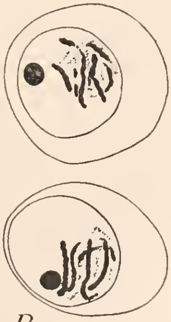


B

12



A



B

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Vol. XII. *March, 1906.* *No. 4*

BIOLOGICAL BULLETIN

STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS

II. Development of the Testes and Spermatogenesis in Moniezia (*Continued*).

C. M. CHILD.

IV. *The Spermatocytic Mitoses.*

The appearance of the chromosomes of the first maturation division follows the stage shown in Fig. 13, *A* (Pl. X.). Figs. 14, *A*, 14, *B*, and 15 (Pl. XI.) show this stage in *M. expansa*, Figs. 16, *A*–16, *C* (Pl. XI.), in *M. planissima*. In the former species eight of these chromosomes have been counted in the nuclei in some twenty-five cases (Fig. 14, *A*) and in no case have more than eight been found. In many cases, however, it has been impossible even with the utmost care in examining successive sections, to find eight, only seven or six (Fig. 14, *B*) being visible. As regards the number in *M. planissima* the results are less definite. In some cases eight (one of the nuclei in Fig. 16, *C*) in others nine (the other nucleus in Fig. 16, *C*) have been counted and in one (Fig. 16, *B*) case thirteen distinct masses of chromatin were visible in one nucleus. This, however, was probably an earlier stage for some of these masses appear to be grouped in pairs. Fig. 16, *A*, shows two nuclei of *M. planissima* in which five of these chromosomes are visible. It was impossible to determine whether parts of these nuclei were in the next section. One reason for the greater uncertainty in regard to *M. planissima* lies in the fact that these stages were much less frequently seen in my sections of this species than in those of *M. expansa*. I believe that the utmost caution should be used in observations of this kind. In a study of cytological literature it is difficult to resist the impression that some of the apparent uniformity in cytological phenomena which appears so remarkable and mysterious is in reality the result of the selection of certain "typical" cases and the discarding of others. While I should not venture to assert positively that the number of chromosomes in either of these species of *Moniezia* is actually variable, this certainly appears to be the case, and the evidence given in support of the conclusion that the number of chromosomes in a given species or variety is invariable has not always carried conviction to my mind.

However, the description of the spermatocytic divisons is not the chief object of the present paper and although I have spent much time in endeavoring to reach well-founded conclusions in regard to the number of chromosomes in *Moniezia* it has not been possible for *M. planissima.* In *M. expansa*, as noted above, the maximal number counted during maturation was eight.

In some of the nuclei at this stage these chromosomes appear, as in many other forms in a more or less regular grouping about the periphery of the nucleus. The figures do not show this condition particularly well but Figs. 14, *A*, 14, *B*, 16, *A*, 16, *C* (Pl. XI.), were drawn from nuclei in which the chromosomes were thus arranged. Such nuclei are recognizable at once in a section. This condition, however, was not very commonly found ; much more frequent was the condition shown in Fig. 15 where they were irregularly disposed, some at the periphery, others near the center. It is possible that the stage of peripheral arrangement is of relatively short duration and is therefore less frequently seen, but there is also a possibility that it is not of universal occurrence. The question may be left open for the present.

In Fig. 17 (Pl. XI.) two of these chromosomes are shown, both from nuclei with membranes still intact. At the stage figured the fusion of the two parts is not yet complete. So far as actual observation goes these chromosomes of the first maturation division are dyads, not tetrads, no indication of quadrivalence having been observed at any stage, and although they correspond as regards ultimate fate with the tetrads of various authors, I prefer to designate them as what they appear to be — dyads. In consequence of their small size, almost spherical form in late stages, and close approximation in the spindle no data regarding the direction of the two divisions could be obtained.

As stated in the preceding section the spermatocytes often appear in groups, the pear-shaped members of which are connected at the pointed end by strands of cytoplasm. As the first spindle forms the fusion of the cells proceeds (Fig. 18, Pl. XI.) until in many cases the metaphases of a number of nuclei lie in a continuous mass of cytoplasm, the cytophore (Fig. 19, Pl. XI.). Often, however, the central region of the mass consists for a considerable time of a large space traversed by strands of cytoplasm (Figs. 18, 20, 23 (Pl. XI.), 25 (Pl. XII.). The strands of cytoplasm are represented in a somewhat diagrammatic manner.) In other cases the first spermatocytes are entirely isolated (Fig. 21, Pl. 11).

The spindle appears to be formed largely from the nuclear substance. The fibers are very delicate and no asters are visible (Figs. 18 and 19, Pl. XI.). At the poles are very minute, but fairly distinct deeply-staining, centrosomes. In the masses resulting from the fusion of several spermatocytes the spindles are, so far as observed, always tangential or nearly so (Figs. 19 and 20, Pl. XI.). Just before their division the dyads assume the form seen in Fig. 22 (Pl. XI.), where one is viewed from the side, the other from the surface. Fig. 20 (Pl. XI.) shows the anaphase. Division of the centrosomes has not been observed, as they cannot be distinguished after the chromosomes have approached the poles. Fig. 23 (Pl. XI.) shows the telophase of the first spermatocytic division in a group. The cytoplasm of the cells which formed the group now constitutes a cytophore in which the masses of chromatin lie. It is less dense in appearance and stains less deeply than in earlier stages, where it was concentrated about particular nuclei. The chromatin masses remain at the periphery and the central region is often still more or less vacuolated. Where isolated spermatocytes divide the result is the same: no division of the cytoplasm follows nuclear division, and a cytophore differing from the group-cytophore merely as regards size is formed.

The second division follows the first, in most cases apparently without the formation of a "resting nucleus" between the two. In a few cases, however, nuclei larger than spermatid nuclei and containing irregular strands and masses of chromatin as if in preparation for a division were formed about the periphery of a cytophore (Fig. 24, Pl. XI.). These were very probably stages following the first spermatocytic division.

Fig. 25 (Pl. XII.) shows the metaphase of the second spermatocytic division in a group cytophore. Here again the divisions are more or less nearly tangential. Fig. 26 (Pl. XII.) shows the anaphase in a portion of a group.

V. *The Fragmentation of Spermatocyte Nuclei.*

In Section III. (pp. 181–182) it was stated that certain of the nuclei of the first spermatocytes become larger than the others, and that the spireme instead of becoming more dense and giving rise to chromosomes becomes less dense and very irregular and the nucleolus remains instead of disappearing (Fig. 13, *B*, Pl. X.). These nuclei constitute the earliest recognizable stage in a remarkable process of fragmentation which is apparently a normal phenomenon in the testes of both species of *Moniezia.* The process is so entirely different from anything described in the spermatogenesis of other forms that at first I regarded it as a form of degeneration. But repeated examination of old and new material during four successive years has convinced me that the process gives rise to nuclei which are indistinguishable from the spermatid nuclei which have arisen by mitosis. Whether these "spermatid nuclei" resulting from the fragmentation of spermatocyte nuclei actually take part in the formation of functional spermatozoa it is impossible to determine, but that spermatozoa structurally similar to those developing from nuclei which arise in the ordinary manner develop from these spermatids is, as will appear probable.

This process, like the typical spermatogenesis, occurs in isolated cells or simultaneously in groups. In the latter case the different members of the group fuse together, in the manner described in the preceding section, and this cytoplasm forms a cytophore. It is rare, however, that groups so regular in form and arrangement as that shown in Fig. 27, *A* (Pl. XII.), are found More commonly only two or three cells fuse together or the process occurs in isolated cells. Fig. 27, *A* (Pl. XII.) shows a group of the nuclei in most of which the spireme is apparently breaking up and disappearing. It stains less deeply than in earlier stages and appears to be separating into irregular masses. Fig. 27, *B* (Pl. XII.), shows an exceptionally clear case, in which some portions of the spireme are apparently breaking up into granules. Figs. 27, *C*, and 27, *D* (Pl. XII.), show still other cases. It will be observed that the nucleolus retains its staining power: as a matter of fact it undergoes no visible change.

Except for the spireme or its remnants the nucleus shows with the usual degree of extraction no visible structural features. As it increases in size the nuclear membrane becomes increasingly difficult to distinguish and finally disappears, so that what was formerly the nucleus appears merely as a cavity in the cytoplasm containing the nucleolus and irregular shreds and granules of chromatic substance usually situated at one side (Figs. 27, *B*, 27, *C*, 27, *D*, 28, Pl. XII.). The whole appears as if degenerating. In these and the following figures the boundaries of the nuclear cavities and the spaces in the cytoplasm which remain in the later stages are indicated by broken lines.

But the observations now to be described afford very strong evidence in favor of the view that these nuclei do not undergo complete degeneration. In and about these cavities containing the remains of the spireme and the nucleolus very small new nuclei with one or a few small granules of chromatin appear to be formed. In Fig. 28 (Pl. XII.), one such nucleus is shown in one of the cells: in Figs. 29, *A*, and 29, *B* (Pl. XII.), two appear in each cell: in Fig. 29, *C* (Pl. XII.), is one a little larger than the preceding; in this case the fragmenting nucleus lies in a cytophore with spermatids already formed and from one of which a spermatozoön is developing: in Fig. 29, *D* (Pl. XII.), are again two of the small nuclei. The nature of the process appears to be somewhat as follows: as the old nuclear membrane breaks down the cytoplasm encroaches more or less upon the nuclear cavity and a new membrane forms about some of the particles or masses of chromatin. This is not very different from what occurs in various forms when a single chromosome or a few chromosomes are separated from the rest during division.

But the new nuclei are not always so small as these shown in

Figs. 28 and 29, *A*–29, *D*. Figs. 30, *A*–*C* (Pl. XIII.), show cases in which they are somewhat larger, and in which the method of their formation is more clearly visible. In these cases the new nuclei are nearly hemispherical in form and appear as if growing or budding out from the cavity representing the old nucleus into the cytoplasm about it. Whether the method of formation is actually the same in all cases is doubtful; sometimes the new nuclei seem to lie wholly within the cavity as in Figs. 29, *A* and 29, *B* (Pl. XII.). But there can be no doubt of their formation. The newly formed nuclear membrane is quite distinct and the whole process presents a very characteristic appearance.

The number of deeply staining bodies visible within these small nuclei depends on the fixation and staining. If extraction is carried beyond a certain point only one dark body retains the stain: otherwise several may be visible, one or two of which are usually larger than the others. After chrom-oxalic the stain is more readily extracted from all except the one body and with the usual degree of extraction only the one body appears. Figs. 29, *A*–29, *D*, are from such preparations. In nearly all cases the nuclei stain somewhat more deeply throughout than the cytoplasm as is indicated in the figures by stippling.

Whether the number of new nuclei formed is always the same is uncertain but it is probable that as many as three or four and perhaps more new nuclei may arise from one old nucleus. In Fig. 29, *E* (Pl. XII.), four small nuclei lie near the lower nuclear cavity but some of these may have arisen from another nucleus.

Figs. 31–36 (Pl. XIII.), show what I regard as later stages in this process. In the group shown in Fig. 31 two spermatocytes were apparently involved and the two old nuclear cavities are still visible, though very irregular in form and divided by strands of cytoplasm. One of the old nucleoli is visible, the other lying in another section. Judging from their position three of the small nuclei were formed about the cavity on the right and one on the other: in the next section more small nuclei were found which probably belonged to the nuclear cavity on the left but in this section other adjoining nuclear cavities with other small nuclei appeared, so that certainty was impossible. It is of interest to note that some of the nuclei in the figure are distinctly hemispherical as if they had been formed in the manner represented in Figs. 30, *A*–30, *C*.

In Fig. 32 (Pl. XIII.) three cavities appear, the old nucleolus being visible in the one on the right. In the middle cavity there are four nuclei. Fig. 33 (Pl. XIII.) shows another case in which portions of two nuclear cavities are visible, each with its nucleolus, and seven nuclei, four about one cavity, three about the other.

In Figs. 34, 35 and 36 (Pl. XIII.) groups of nuclei are shown lying in cavities of the cytophore : nucleoli were not visible in this section and it was impossible to determine how many spermatocytes were involved in the formation of these groups. That such groups as these are formed by fragmentation there can, I think, be no doubt. In all cases where the spermatocytes divide mitotically in groups the spindles lie peripherally and nearly tangentially in the cytophore (Figs. 19, 20, 23, Pl. XI.; 25, 26, Pl. XII.), and the nuclei formed lie at or near the periphery (Figs. 24, Pl. XI.; 42, 43, 44, Pl. XIV.). There are no grounds for supposing that they migrate from the periphery and return to it ; moreover, it is difficult to see how nuclei grouped as those in Figs. 34 and 35 (Pl. XIII.) are grouped could have been formed *in situ* by mitosis. It seems probable that this difference in position is quite sufficient to distinguish the nuclei formed by fragmentation of spermatocytes from those formed by the spermatogenetic mitoses.

Assuming that these nuclei have arisen by the fragmentation of spermatocytes, the number of chromatin granules has increased beyond that contained in some of the nuclei at the time of their formation and the nuclei are now somewhat larger than some of those. No further growth takes place, however. The nuclei themselves are indistinguishable from those spermatid nuclei which lie at the periphery of the cytophores and are doubtless the products of mitoses (Figs. 42, 43, Pl. XIV.).

Figs. 37 (Pl. XIII.) and 38 (Pl. XIV.) represent larger areas of two testes showing how the nuclei which, judging from their position in the cytophore, have arisen by fragmentation are situated in relation to other stages. No trace of definite position or arrangement of the various stages in the testis could be found.

But these nuclei apparently do not remain massed together in the cytophore. There is every reason to believe that they migrate to the periphery. Very frequently cytophores with several old nucleoli imbedded in different regions of their cytoplasm, indicating that fragmentation has occurred, show all their nuclei at the periphery as in the case of spermatids formed by mitosis. Since I have never found first spermatocytic mitoses and fragmentation occurring in the same cytophore and since the nucleolus disappears before mitosis it seems probable that the presence of the old nucleoli which stain very characteristically and are readily recognizable is sufficient to identify a particular cytophore and its spermatid nuclei, for such, I believe we may call them, at least so far as appearance goes, as the result of fragmentation.

Fig. 39 (Pl. XIV.) is probably a stage in the migration to the periphery of spermatid nuclei formed by fragmentation. At the right of the figure one of the old nucleoli, itself near the periphery, and a part of an old nuclear cavity containing a few granules are visible: another lies near the middle of the cytophore and still others were found in the same cytophore in other sections. Some of the nuclei have already reached the periphery of the cytophore and the cytoplasm about them bulges from its surface. Most of these nuclei are more or less hemispherical with convex surface toward the periphery and a space adjoins the flattened side: this condition is probably connected with the migration of the nuclei toward the periphery. Indeed, it is not impossible that this migration may not be a bodily movement through the cytoplasm of the cytophore, but rather the continued formation of new nuclear membrane on the peripheral side and its continued disappearance on the proximal side. In any case the change of position is probably a "tactic" reaction of some sort.

In two testes cytophores have been found containing nuclei intermediate in size between those of the spermatocytes at the time of fragmentation and those of spermatids, but which appeared as if preparing for fragmentation. Fig. 40 shows one of these cases. These two groups may possibly be cases of secondary fragmentation. Occasionally as in Fig. 30, *A* (Pl. XIII.), the nuclei arising by fragmentation are unusually large and perhaps undergo fragmentation again. At any rate such cases are rare and not of fundamental importance. In one case a mitotic spindle was observed in a cytophore whose nuclei had apparently arisen by fragmentation since two nucleoli were present and the nuclei were not at the periphery (Fig. 41, Pl. XIV.). This would appear to be a case where a nucleus resulting from fragmentation divides mitotically afterward: it might, however, be a case where a spermatocyte undergoing mitosis had fused with a group undergoing fragmentation: if that were the case the size of the spindle would seem to identify it as the second spermatocytic division. But whatever its interpretation, this, too, is clearly an exceptional case. It is probable that a large proportion of the nuclear substance of the spermatocyte passes into the cytoplasm at the time of fragmentation. It may be that a kind of reduction is accomplished in this manner.

The relative frequency of fragmentation and mitosis seems to vary in different chains, proglottids and testes. In some chains the spermatogenetic mitoses were rarely seen except in the older proglottids, yet spermatozoa were produced in the younger proglottids as abundantly apparently as elsewhere. In some other cases mitosis is more frequent. From examination of the testes one gains the impression that the mitoses are not in any case sufficiently numerous to account for the large number of spermatids and spermatozoa formed. I regard it as at least probable that spermatozoa are produced from the "spermatid" nuclei which arise by fragmentation, as well as even those which arise by mitosis. As will appear in a later section, some cells undergo degeneration in almost all or all testes and it is of course impossible to prove that these particular spermatid nuclei which arise by fragmentation do not undergo degeneration. Still, developing spermatozoa have been found on cytophores containing the old spermatocyte nucleoli.

VI. *The Formation of the Spermatozoön from the Spermatid.*

In consequence of the difficulty of observation of details in these exceedingly minute structures, which is farther increased by the massing together of the spermatids in cytophores and the condensation of these in later stages, it has not been possible to reach positive conclusions on all points. It can scarcely be doubted from my own observations as well as those of others that the development of the spermatozoön in these forms differs in certain respects from the typical method. Although the greatest caution has been observed throughout, the observations are given with a certain reserve, because it was impossible to attain complete certainty on many points and because they are not in accord with commonly accepted opinions regarding the development of the spermatozoön. I believe, however, that a careful investigation of spermatogenesis in the cestodes will prove of interest.

Except in the early stages when the spermatid nuclei produced mitotically appear in pairs about the cytophore (Fig. 42, Pl. XIV.) and those arising by fragmentation of the spermatocytes are massed in the interior of the cytophore (Figs. 34–36, Pl. XIII.) there is no certain criterion for distinguishing the two kinds. The presence of old nucleoli in a cytophore render it probable that all or a part of the spermatid nuclei of that cytophore have arisen by fragmentation, but beyond this no means of identifying the nuclei of different origin has been discovered.

The following account concerns the spermatids without respect to origin. If my conclusions are correct, however, many of these may be the results of fragmentation. As was noted above, developing spermatozoa not different in appearance from others have often been found on cytophores containing the old nucleoli.

Figs. 42 and 43 (Pl. XIV.) show the newly formed spermatid nuclei after mitosis. In Fig. 43, from *M. planissima*, five chromatin granules are distinctly visible in each of the nuclei. These may represent five chromosomes: it is possible that the spermatocytes of this species contained only five dyads (Fig. 16, *A*, Pl. XI.) and that the cases where a larger number seemed to be present (Figs. 16, *B*, 16, *C*, Pl. XI.) were only earlier stages before the chromatin had become massed in the dyads.

After the formation of the spermatid nuclei their peripheral position on the cytophore becomes more and more marked until finally each is borne on a short peduncle or stalk of cytoplasm (Fig. 44, Pl. XIV.). The cytophores differ greatly in size according as they were formed from a single spermatocyte or a larger number: in fact in older testes single isolated spermatids are sometimes found which have apparently become entirely separated from the cytophore.

The spermatid nuclei contain at this time only a few very distinct deeply staining granules (some of the nuclei in Fig. 44, Pl. XIV.): in cases where extraction is carried to extremes only two granules, one at the peripheral end of the nucleus, the other near the middle or at one side of the nucleus (some of the nuclei in Fig. 44, Pl. XIV.). The peripheral granule is closely applied to the nuclear membrane, so closely indeed that it is often difficult to determine whether it is inside or outside the nucleus. In some cases, however, it is clearly inside the nucleus (Fig. 44, Pl. XIV.) and this is probably its position in all cases.

The first visible step in the formation of the spermatozoön is the appearance at the periphery of the cytoplasm peripheral to the nucleus of a minute deeply staining granule. In position this granule corresponds to the peripheral centrosome which enters the middle piece in the spermatozoa of many other forms. It has been impossible in consequence of the small size of these cells to obtain any data regarding its origin in this case. If the spermatids arising by fragmentation do produce spermatozoa the question as to its origin in those cases is of some interest. This peripheral body which apparently lies in contact with the border of the cytoplasm appears to be connected by a very delicate cytoplasmic strand or fiber with the granule at the peripheral end of the nucleus (Figs. 44 and 45, Pl. XIV.). Whether there is another cytoplasmic granule in contact with or near the nucleus corresponding in position to the other centrosome of other forms could not be determined. From the peripheral granule in the nucleus the delicate fiber appears to continue through the nucleus usually to the second granule (some of the nuclei in Fig. 44, also Fig. 45, Pl. XIV.). This continuation of the fiber within the nucleus has been a matter of the most careful examination and I can say regarding it only that I have seen it in the nuclei of practically every cytophore examined and under the most various conditions of fixation and staining so that if present methods of technique permit trustworthy conclusions in regard to such matters its existence seems beyond doubt. The figures exaggerate its distinctness to some extent. It does not stain as deeply as the granules themselves but this is very likely due to its smaller diameter.

The next step is the formation of the tail which appears first as a delicate thread extending from the granule at the border of the cytoplasm (Fig. 46, Pl. XIV.).

Figs. 47, *A*–47, *E* (Pl. XIV.) show the developing spermatozoa after different methods of fixation and staining : Fig. 47, *A*, is from *M. expansa* after sublimate and Delafield's hæmatoxylin ; Fig. 47, *B*, *M. expansa* after sublimate and iron-hæmatoxylin ; Fig. 47, *C*, *M. planissima*, after chrom-oxalic and iron-hæmatoxylin ; Fig. 47, *D*, *M. expansa*, after Hermann and iron-hæmatoxylin. Fig. 47, *E*, is from *M. planissima* after sublimate and iron-hæmatoxylin, but with extraction stopped at an earlier stage. One interesting point in this figure as compared with the others is the much larger size of the peripheral cytoplasmic granule and the fiber connecting it with the nucleus — an excellent illustration of the uncertainty attending the use of iron-hæmatoxylin.

The tail of the spermatozoön grows to a very great length. Fresh spermatozoa obtained by teasing living proglottids in indifferent fluids are 0.3–0.4 mm. in length. Most or all the tails arising from one cytophore usually lie parallel in the testis, and since their length is much greater than the diameter of the testis they become coiled in the spaces between the cells or along the wall of the testis. The tail is very delicate and without visible differentiation in structure.

As regards the formation of the head of the spermatozoön *Moniezia* does not seem to agree with other species described. At least I know of no other case in which the sperm-head, if it can be called a head, is formed in the manner described below.

In my study of spermatogenesis I was for a long time puzzled by the fact that all of the sperm nuclei appeared to degenerate after the tails were formed. Masses like Fig. 49 (Pl. XV.) consisting of degenerating nuclei and condensed cytophore cytoplasm can be found in every older testis. At first I concluded that these were probably the spermatids formed by fragmentation which began the development of spermatozoa but were unable to complete it. But another feature made the matter still more puzzling. The most careful examination, under varied conditions of fixation and staining, of spermatozoa in the male ducts and in the seminal receptacle of the female ducts, which becomes greatly distended with them at a certain stage, failed absolutely to reveal the existence of a head differing in appearance from the tail. The examination and staining of fresh spermatozoa from the seminal receptacle and ducts of living proglottids led to the same result. The spermatozoa appeared as very long thread-like structures perhaps slightly larger at one end than at the other but without the least trace of a physically or chemically differentiated head.

Then the question arose as to whether the eggs were actually fertilized by these spermatozoa. As will be described in the following paper, the spermatozoa were found entering the eggs as these passed the opening from the seminal receptacle on their way to the uterus, and nuclei which could be nothing else than male pronuclei unless these eggs differ from other known cases in their maturation and fertilization stages were found. Returning to the developing spermatozoa the most careful study was made of the various stages and especially of the masses like Fig. 49 (Pl. XV.) which were apparently undergoing degeneration. It is very difficult to distinguish details in these masses for they stain more deeply as they condense and the nuclei especially become more or less filled with deeply staining granules and masses. In the course of time certain apparently favorable cases were found some of which are shown in Figs. 48, *A*–48, *C* (Pl. XV.). These seem to indicate that the "head" of the spermatozoön, *i. e.*, the part arising from the nucleus is formed from the two nuclear granules, the peripheral and the other which may be central or proximal, together with the connecting strand, and furthermore, that when degeneration of the other parts of the nucleus begins the spermatozoön is set free. Figs. 48, *A*–48, *C*, show examples of the early stages of nuclear degeneration in sperm cytophores. In Fig. 48, *C*, the spermatozoön head is apparently in the act of escaping from the degenerating nucleus. The peripheral portion of the nuclear membrane has disappeared but the peripheral nuclear granule is still recognizable. Figs. 48, *A*, and 48, *B*, are apparently somewhat earlier stages in which the nuclear membrane is still intact. I am forced therefore to the conclusion that only a part of the nucleus is concerned in the formation of the sperm-head, the remainder undergoing degeneration. Fig. 49 (Pl. XV.) represents a cytophore after condensation. For the sake of clearness only a few of the nuclei which cover the surface of the mass are represented as they appear, the others being indicated by dotted lines. In several cases what seems to be the sperm-head is visible in the degenerating nucleus.

Fig. 50 (Pl. XV.) represents a case in which the spermatozoa are apparently just separating from the cytoplasm of the cytophore which contains the deeply staining remains of the nuclei. In this case I convinced myself that these were the anterior ends of the spermatozoa, by following the tails throughout their whole length in the testis. The diameter of the ends shown in the figure was distinctly though only slightly greater than that of the other ends, but the change in diameter is very gradual.

In the free spermatozoa no differentation in staining of the head-region is visible. The whole spermatozoön stains uniformly and less intensely than the nuclear granules or masses of earlier stages. In a few cases I believed I had distinguished slight traces of the two nuclear granules in the fully developed spermatozoön but these observations were so doubtful that no figures are given.

In the fresh spermatozoa obtained by teasing in indifferent fluids no visible head and no movement was ever observed. An examination of the bibliography of the subject afforded scanty results. So far as I have been able to determine no full account of the spermatogenesis of the cestodes exists. Among the older papers several give brief descriptions of the formation of the spermatozoa but these are either very incomplete or incorrect in consequence of the technique employed and need not be reviewed in detail. In one point, however, the early observations agree fairly well : the head of the spermatozoön is described and figured as exceedingly minute or is said to be absent. Sommer and Landois[1] in describing the testes of *Bothriocephalus latus* mention spermatozoa bearing at one end "ein kleines, stark lichtbrechendes Köpfchen."

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[1] Sommer and Landois, "Ueber den Bau der geschlechtsreifen Glieder von *Bothriocephalus latus* Bremser," *Zeitschr. f. wiss. Zoöl.*, Bd. XXII., 1872.

Two years later Salensky[1] states that in *Amphilina* the nuclei disappear completely in the formation of the spermatozoa and that "die Kerne bei der Bildung der Spermatozoen keine Rolle spielen." Regarding the fully formed spermatozoa he says: "Die Fäden sind sehr lang, ungefahr 0.27 mm. und an einen Ende etwas gekrummt. Diese Krümmung soll aber nicht als Köpfchen angesehen werden, indem die spermatozoen in ihrer ganzen Länge gleich dick sind."

As regards *Tænia mediocanellata* and *Tænia solium* Sommer[2] speaks of the bundles of spermatozoa which hang from certain large cells (in reality the cytophores) and "mit ihren äusserst feinen, glänzenden Köpfchen noch in Zellenprotoplasma stecken." These "Köpfchen" are probably the nuclear granules which present this appearance in unstained or slightly stained preparations, the nuclear membrane not being clearly visible. "Zwischen diesen Samenfäden producirenden Zellen findet man gleichzeitig im Hodenkörperchen kleine Anhäufungen freier, heller, scharf contourirter und bläschenförmiger Kerne. Einzelne derselben haben an ihrem Grenzrande noch Spuren von Protoplasma in welchen mit seinem glänzenden punctförmigen Köpfchen ein Samenfädchen haftet." These masses are perhaps degenerating cytophores. In another paragraph he describes the formation of the large multinucleate cells which give rise to the spermatozoa and says: "An der Peripherie dieser grossen Zellen geht von irgend einer Stelle die Bildung der Samenfäden aus. Letztere entstehen lediglich aus dem Protoplasma der Zelle; eine Betheiligung der Kerne dabei findet nicht statt. In demselben Maasse wie mit der Bildung der Samenfäden das Protoplasma der Zelle schwindet, werden die eingelagerten Kerne frei, erscheinen dann schärfer berandet wie früher, etwas aufgebläht oder gequollen, homogen und wasserhell, dann fallen sie zusammen, collabiren, wie wenn sie einen flussigen Inhalt entleert hatten und gehen zu grunde, oder werden, wenn sich inzwischen Samengänge ge-

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[1] Salensky, "Ueber den Bau und die Entwickelungsgeschichte der *Amphilina*, G. Wagen (*Monostomum foliacium* Reed)," *Zeitschr. f. wiss. Zool.*, Bd. XXIV., 1874.

[2] Sommer, "Ueber den Bau und die Entwickelung der Geschlechtsorgane von *Tænia mediocanellata* (Küchenmeister) und *Tænia solium* (Linné)," *Zeitschr. f. wiss. Zool.*, Bd. XXIV., 1874.

bildet haben mit den Samenfäden fortgespielt." Sommer's preparations were obtained by maceration and teasing and without staining. As described, the fate of the nuclei does not differ very widely from that described in the present paper except for the fact that Sommer failed to observe that any portion of the nucleus took part in the formation of the spermatozoön. Considering the methods employed this failure is not strange.

Moniez[1] describes the formation of large multinucleate cells and the protrusion from their surface of the nuclei which are united with the body of the cell by pedicels (these are evidently the spermatids on the cytophore). He continues as follows: "Ces nouvelles formations qui rayonnent de la cellule-mere sont les vrais spermatozoides : leur flagellum se forme à la partie périphérique, tandis qu'ils sont encore fixé par l'autre extrémité ; c'est apres qu'ils se sont détachés que leur tete s'atrophie comme l'on sait." These facts he describes as common to a number of species among them *Tænia expansa, i. e., Moniezia expansa* as it is now known.

In describing the spermatozoa of *Tænia saginata* Leuckart[2] speaks of the "freilich kaum ausgezeichneten" head.

From all of these observations it is evident that where a distinct head is visible it is exceedingly minute and the observations of Salensky, Sommer, and Moniez seem to indicate that the spermatozoa of several species are without visible heads. The description of the collapse and degeneration of the nuclei by Sommer and the mention of atrophy of the head by Moniez appear to be somewhat closely in line with my own observations. But until other species have been examined with the aid of present cytological methods general conclusions are impossible. I am convinced, however, that if the spermatozoa of *Moniezia* possessed distinct, visibly differentiated heads I should have seen them in some cases at least. Comparative study of other species will undoubtedly prove of interest.

VII. *The Degeneration of Cell-Groups in the Testis.*

During almost the whole period of existence of the testis groups of cells undergo degeneration from time to time. Cells in any stage of development from the spermatogonia to the developing spermatozoa except during the spireme stage are subject to this fate. The proportion of degenerating cell-groups varies greatly in different chains, proglottids and testes. In some chains only one or two cases of degeneration preceding the first appearance of the spireme stage have been observed. In others degenerating groups are found in almost every testis in the spireme period. During the earlier stages of the process the degenerating cells form rounded masses: later these break up and become distributed through the testis and are apparently absorbed by the cytoplasm of other cells. While it is impossible to assign positively a definite reason for this degeneration I am inclined to believe that it results from differences in physiological condition which may in turn be correlated with differences in nutrition. Careful examination of regions of rapid growth in many forms often shows a certain proportion of cells which are undergoing degeneration. Undoubtedly in such regions the intensity of certain stimuli or conditions carries some cells beyond the point where physiological equilibrium can be regained and they degenerate, serving perhaps as food for the others. There can be little doubt that the testis is a region of this sort. The great variation in the frequency of degeneration in different chains may indicate that it is connected with nutritive conditions. Apparently more cells are produced than can be sustained and some are eliminated.

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[1] Moniez, "Sur les Spermatozoides des Cestodes," *Comptes Rendus*, 1878.

[2] Leuckart, "Die Parasiten des Menschen," 1879–1886.

The fact that no case of degeneration beginning during the spireme stage has been observed may be of some interest. It is not improbable that this stage is relatively independent of external conditions, *i. e.*, that a cell having entered this stage is capable of completing it without the intervention of external factors. To judge from appearances this stage is a readjustment or the establishment of a new condition of equilibrium in the cell and it may represent a reaction from previously existing conditions which have disturbed the previous equilibrium of the cell. There can be little doubt that in many respects the life of the cell possesses a cyclical character. One complex of processes or reactions continues until it brings about a reversal in reaction or initiates a different complex, etc.

That this degeneration has any connection with amitosis is extremely improbable. In no case has a whole testis been found undergoing degeneration, yet in all the testes most of the divisions before spermatogenesis proper were amitotic and in the great majority the first divisions certainly were amitotic. As was suggested in the preceding section degeneration of cell-groups in post-spireme stages may be connected with the fragmentation of spermatocyte nuclei though this seems improbable, and moreover, it does not explain degeneration in pre-spireme stages. I believe, though I see no way of demonstrating it, that the method of origin of the cell-groups in the testis has no connection with their degeneration.

The degenerating cell-groups vary greatly in appearance according to the stage at which degeneration begins and the different stages of degeneration itself. In many cases, though not always, it is possible to determine from the appearance of the degenerating mass approximately the stage at which degeneration began. In some cases cells in the same stage of development undergo two different processes of degeneration.

Some of the characteristic forms and stages of degenerating cell groups are shown in the following figures : in these figures no attempt has been made to represent the cytoplasmic background. This varies somewhat in density and staining in different cases. Vacuoles and spaces are indicated by broken lines. The method of reproduction exaggerates the depth of shade in the more deeply staining portions. Fig. 51 (Pl. XV.) shows a small group of cells from a young testis in the first stages of degeneration. The first evidence of degeneration in these cases is a condensation of the cytoplasm and a massing together of the nuclei, and the degenerating group becomes quite distinct from other cells, usually lying in a space. Fig. 52 (Pl. XV.) shows a later stage of this form of degeneration ; the nucleoli increase in size and stain very deeply, the nuclear membrane becomes indistinct, and the whole mass stains more intensely. Later, as shown in Fig. 53 (Pl. XV.), the mass breaks up into irregular deeply staining fragments and strands which are distributed through the testis and are often found in the cytoplasm of other cells surrounded by small vacuoles ; a few of these fragments in the cytoplasm are shown in the figure.

Fig. 54 (Pl. XV.) represents a form of degeneration in cells in prespireme stages which sometimes occurs in old testes. Here the nuclei form irregular densely staining masses and finally the whole breaks up and is absorbed.

Fig. 55 (Pl. XV.) shows degeneration of a group of spermatid nuclei in a cytophore. The deeply staining granules and masses in the nucleus increase in number, the nuclear membrane breaks down, and the granules are distributed through the cytoplasm. In Fig. 56 (Pl. XV.) another form of spermatid degeneration is seen and a later stage in Fig. 57 (Pl. XV.). Fig. 58 (Pl. XV.) represents a still later stage : vacuoles usually containing a single granule still indicate the position of the nuclei ; the mass stains only very faintly at this stage and seems to decrease gradually in size until finally it becomes imbedded in a cytophore and gradually disappears.

Fig. 59 (Pl. XVI.) represents a form of degeneration of the spermatids which usually occurs only after the spermatozoa have begun to develop. About the periphery of each nucleus a large amount of deeply staining substance develops and appears to flow toward the center of the cytophore. In Fig. 60 (Pl. XVI.) is shown a later stage of this form of degeneration. Here the cytoplasm stains rather more deeply than that of the normal cytophore, the deeply staining substance has disappeared entirely from the peripheral regions except in a few radiating strands and the positions of the nuclei are indicated only by vacuoles. In still later stages (Fig. 61, Pl. XVI.) the deeply staining substance gradually breaks up into granules (Fig. 64, *k*, Pl. XVI.), loses its staining power and finally disappears, and the whole cytophore becomes highly vacuolated, breaks up into irregular masses and shreds (Fig. 64 *l*, Pl. XVI.) and is apparently absorbed.

Other modifications of the process of degeneration are occasionally seen but these are the principal ones. The apparent variation which these processes exhibit is of some interest as indicating that differences in the processes of degeneration like differences in development are undoubtedly determined by differences in the condition of the cells or of the environment. At present, however, even a surmise as to the nature of these differences is of little value.

VIII. *The Full-Grown Testis.*

The testis continues to increase in size for a considerable time after spermatogenesis begins. Only a part of the spermatogonia enter the spireme stage at any one time, the others continuing to divide amitotically. After the appearance of the spermatogenetic divisions in a testis, I have never seen a case of mitosis in the spermatogonia, but amitoses are frequent. Figs. 62, *A*, 62, *B* (Pl. XVI.) represent groups of spermatogonia in full grown testes. In the same testes all stages of spermatogenesis and fully developed spermatozoa may be found. At this period the spermatogonia are usually found in small groups near the periphery. Figs. 63, *A*–63, *D* (Pl. XVI.), show cases of amitosis in spermatogonia from full grown testes, including nearly all the modifications of the process observed.

In Fig. 64 (Pl. XVI.), one half of a full-grown testis is shown on a scale half as large as that of the other figures. The different stages shown are as follows: at *a* is a group of spermatogonia still in the prespireme stage and showing one amitosis; *b* shows the earliest stages of the spireme, *c*, *c*, two groups with fully-developed spireme, while at *d* some cells are preparing for the first spermatocytic mitosis; at *e* is a cell in which the dyads are formed, part of a group which appears in adjoining sections; *f*, *f*, are cytophores with spermatid nuclei and developing spermatozoa; *g*, is a cytophore in which degeneration of the nuclei has begun, but the spermatozoa are still attached; at *h* is seen part of a bundle of free spermatozoa which can be followed in other sections; *k*, *k*, represent two degenerating cytophores in which the nuclei have already vanished: the shreds of cytoplasm and the debris from earlier cytophores are indicated at *l*. Although this one section does not show all the stages in the history of the cells, it serves to indicate the promiscuous distribution of different stages.

IX. *Conclusion.*

The point of chief importance in the present paper is the fact that typical mitosis and amitosis may appear together and apparently under identical conditions in the development of the male as well as of the female germ cells. The relative frequency of the two forms of division varies in different chains, proglottids and regions. Observations and experiments to be described later will show very clearly, however, that amitosis as well as mitosis is an important factor in growth, not only in *Moniezia* but in many other forms and that in some cases at least either form of division may be changed into the other by altering the conditions.

These facts are of considerable importance as bearing upon certain hypotheses regarding the significance of the chromosomes. At present it seems improbable that the views held by certain authors regarding the individuality of the chromosomes can be reconciled with them. Extended discussion is, however, postponed until other facts have been presented.

The most important features in the development of the male germ cells in *Moniezia* are as follows :

The testes apparently arise from cells which are already differentiated as muscle-cells, as well as from other cells of the parenchyma. The earlier divisions are almost entirely amitotic, mitosis being rarely seen.

The growth of the testis up to the time when spermatogenesis proper begins is almost wholly by amitotic division. In the full-grown testis the remaining spermatogonia still continue to divide amitotically. After the spireme stage the spermatocytes follow two very different lines of development. In some of them typical dyads are formed and the two usual spermatogenetic mitoses follow : the spermatid nuclei are usually situated about the periphery of large masses of cytoplasm, cytophores formed by fusion of the spermatocytic cytoplasm, but may be isolated.

In the other spermatocytes the nucleus increases in size, the spireme breaks up into granules and masses and loses most of its staining power, the old nuclear membrane disappears, and new nuclear membranes form about small fragments of the chromatin : each spermatocyte may give rise to several small nuclei : in appearence these nuclei are indistinguishable from the spermatid nuclei produced mitotically. When first formed they are massed in groups in the interior of the cytophore about spaces which indicate the former positions of the spermatocyte nuclei. The nucleolus does not take part in this fragmentation but remains in the cytoplasm of the cytophore for some time. The nuclei thus formed gradually make their way to the periphery of the cytophore and probably give rise to spermatozoa, though this cannot be demonstrated with absolute certainty.

Apparently only a part of the nucleus is involved in the formation of the anterior end of the spermatozoön in which no "head" is visible. The sperm-head is apparently represented by two granules in the nucleus, one peripheral, one more or less nearly central and a less deeply staining fiber which connects them, these being in most cases the only deeply staining portions of the nucleus. When development of the spermatozoön is completed the nuclear portion is apparently set free from the remainder of the nucleus by the degeneration of the latter.

Groups of cells in all stages of development except the spireme stage are frequently attacked by degenerative processes probably because of insufficient nutrition or exhaustion.

Hull Zoölogical Laboratory,
University of Chicago,
September, 1906.

EXPLANATION OF PLATES.

PLATE XI.

FIG. 14. *A*, *B*, the dyads grouped about the periphery of the nucleus. *M. expansa.*

FIG. 15. Dyads irregularly disposed in nucleus. *M. expansa.*

FIG. 16. *A–C*, dyads. *M. planissima.*

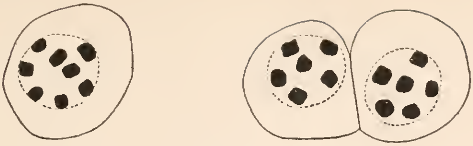
FIG. 17. Two dyads more highly magnified.

FIGS. 18, 19, 20, 21. Different stages of first spermatocytic mitosis.

FIG. 22. Two dyads in metaphase, more highly magnified.

FIG. 23. After the first spermatocytic division.

FIG. 24. Probably resting nuclei after first spermatocytic mitosis.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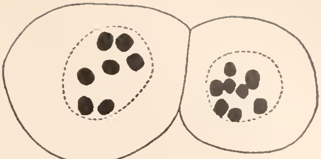
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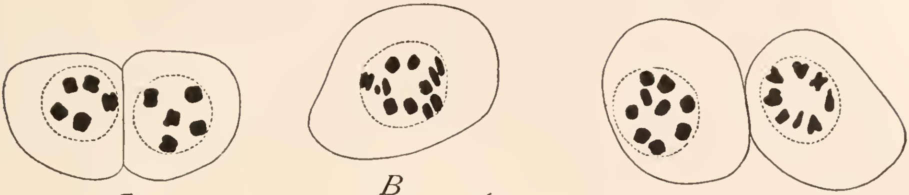
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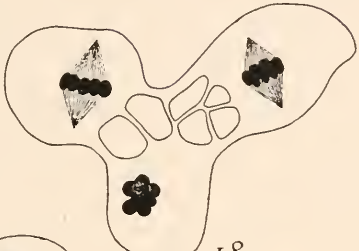
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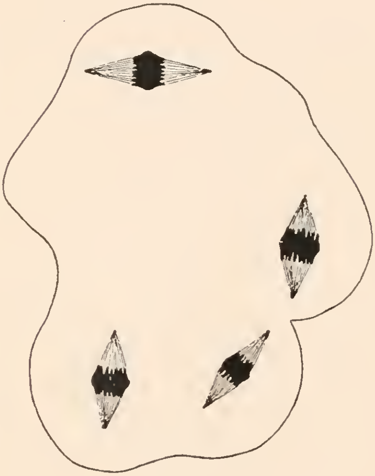
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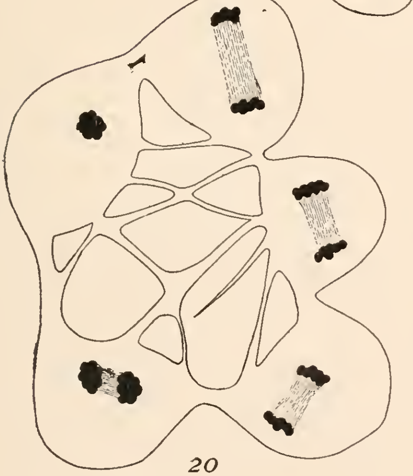
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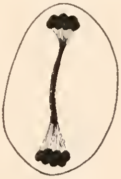
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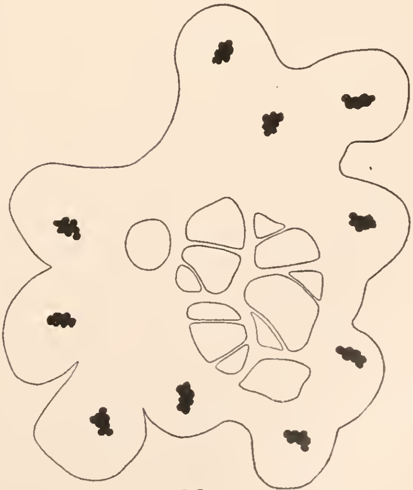
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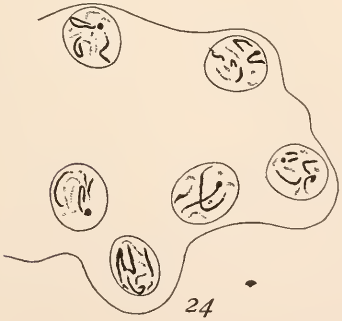
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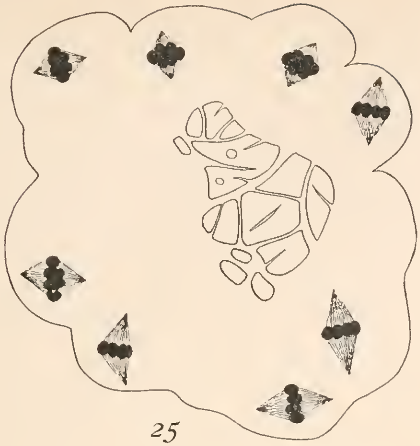
Plate XII.

Figs. 25, 26. The second spermatocytic mitosis.

Fig. 27. *A–D*, early stages of fragmentation of nuclei of first spermatocytes.

Fig. 28. Fragmentation. One small nucleus forming.

Fig. 29. *A–E*, the formation of nuclei by fragmentation.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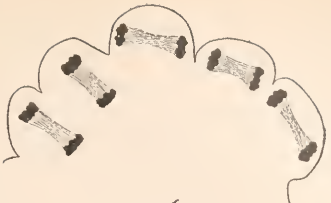
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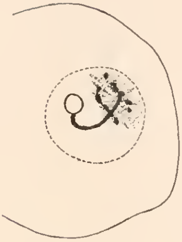
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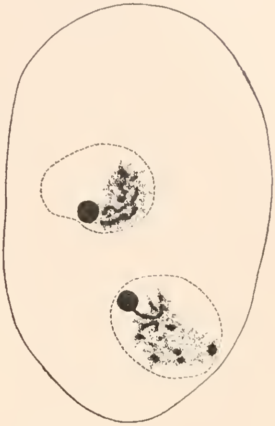
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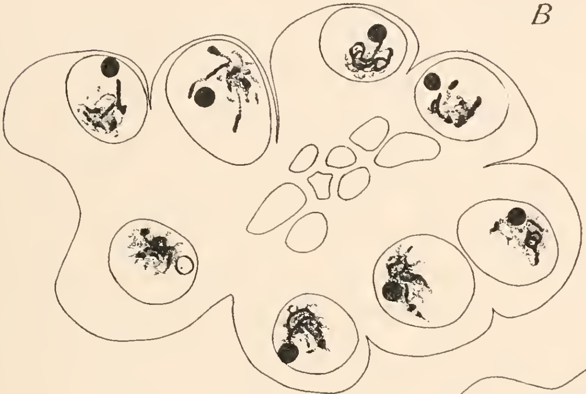
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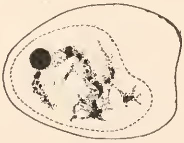
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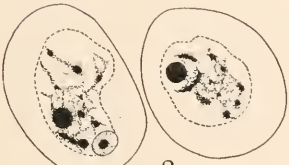
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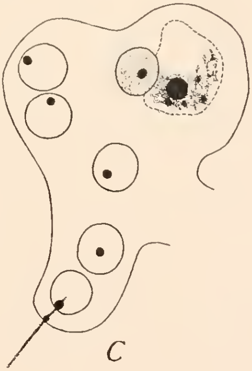
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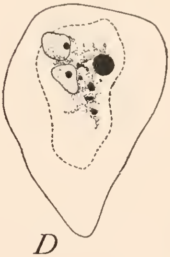
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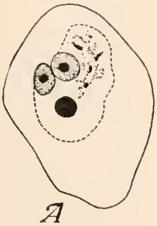
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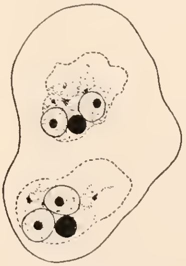
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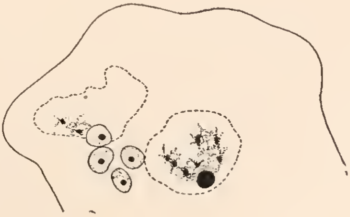
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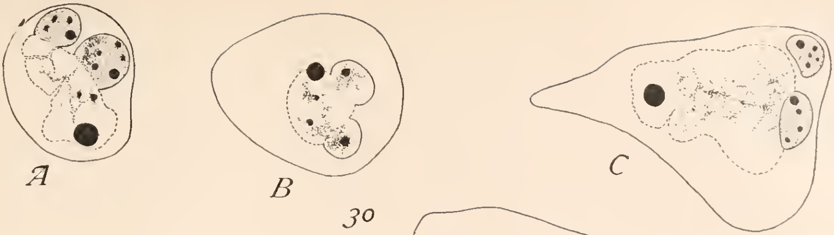
PLATE XIII.

FIG. 30. *A–C*, the formation of nuclei by fragmentation.

FIGS. 31–36. Nuclei and old nucleoli in the cytophores after fragmentation.

FIG. 37. Nuclei and old nucleolus after fragmentation with spireme stages adjoining.



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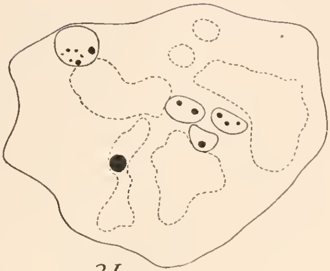

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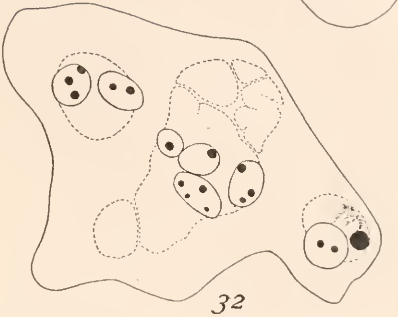

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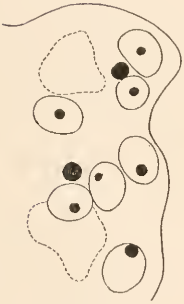

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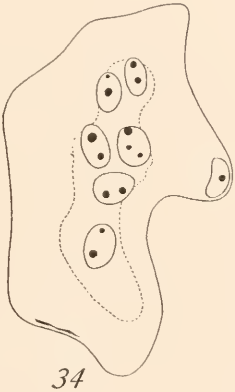

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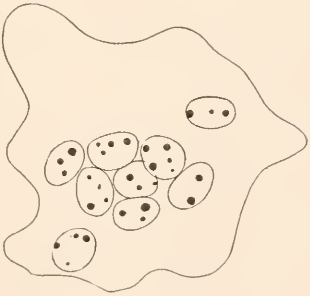

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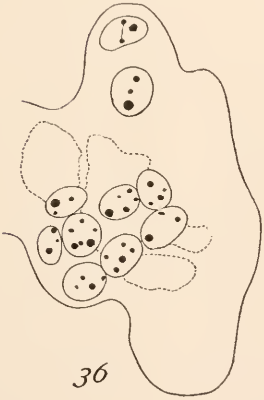

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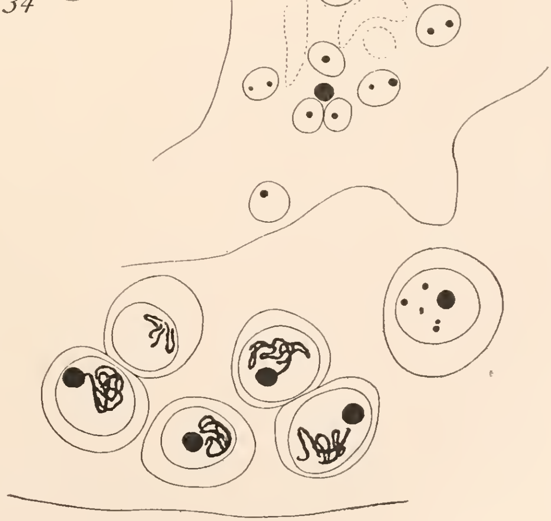

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PLATE XIV.

FIG. 38. Nuclei and old nucleolus after fragmentation with other stages adjoining.

FIG. 39. Probably migration of nuclei to periphery after fragmentation.

FIG. 40. Possible case of secondary fragmentation.

FIG. 41. A case of mitosis in a cytophore which was probably formed by fragmentation.

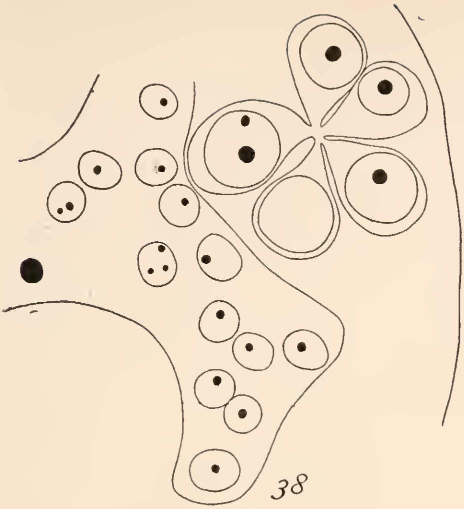
FIG. 42. Spermatid nuclei formed by mitosis.

FIG. 43. Spermatid nuclei.

FIGS. 44 and 45. Early stages in the development of the spermatozoa.

FIG. 46. Development of the spermatozoa.

FIG. 47. *A–E*, development of the spermatozoa after different methods of fixation and staining; *A*, *M. expansa*, sublimate and Delafield's hæmatoxylin; *B*, *M. expansa*, sublimate and iron-hæmatoxylin; *C*, *M. planissima*, chrom-oxalic and iron-hæmatoxylin; *D*, *M. expansa*, Hermann and iron-hæmatoxylin; *E*, *M. planissima*, sublimate and iron-hæmatoxylin, extraction stopped at an early stage.



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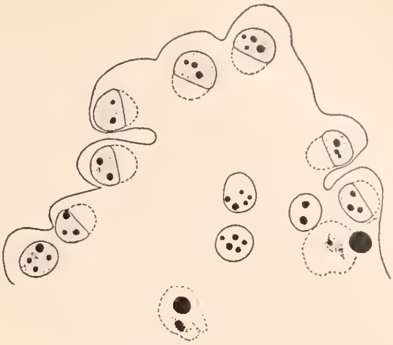

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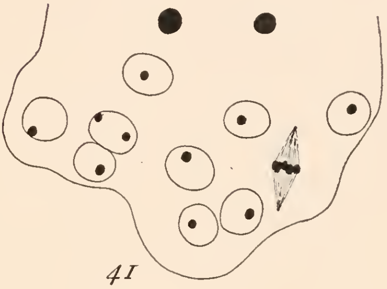

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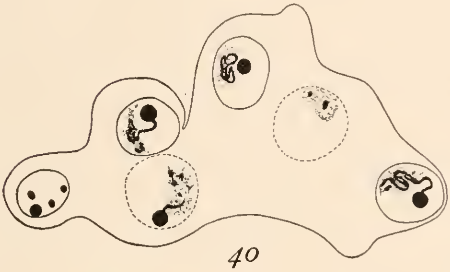

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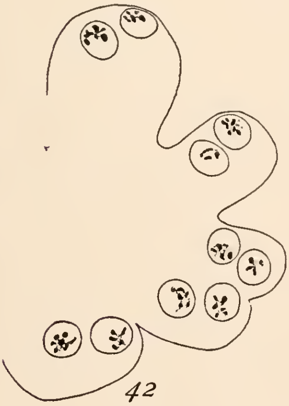

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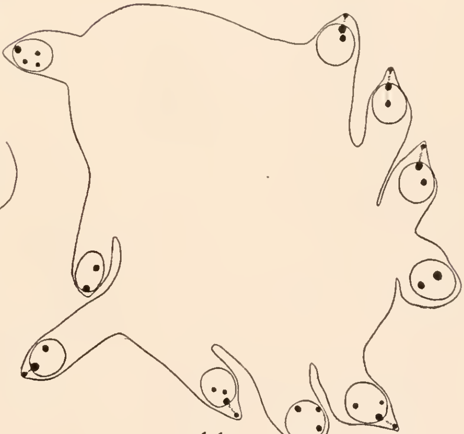

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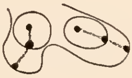

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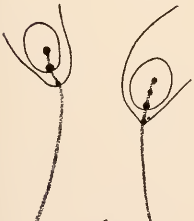

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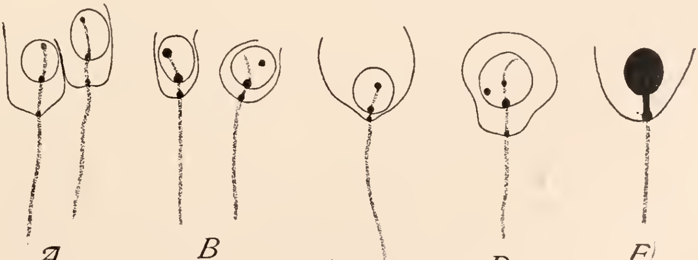

A B C D E

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PLATE XV.

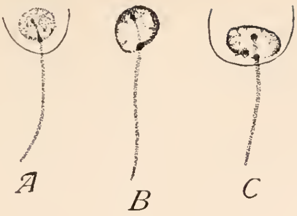
FIG. 48, *A–C*. The spermatozoön and the degenerating portions of the spermatid nucleus.

FIG. 49. A cytophore with spermatozoa and degenerating spermatid nuclei.

FIG. 50. Spermatozoa becoming free from degenerating cytophore.

FIGS. 51–54. Degeneration of cell groups in prespireme stages.

FIGS. 55–58. Degeneration of cell groups in spermatid stage.



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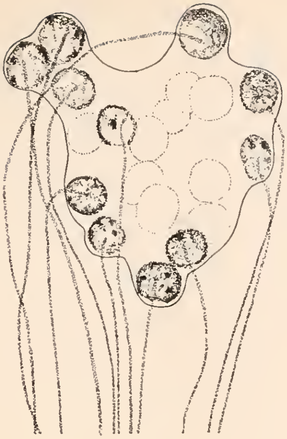

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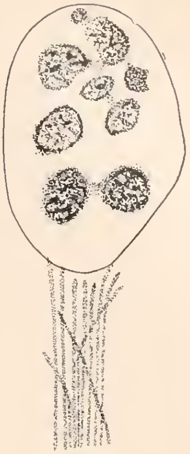

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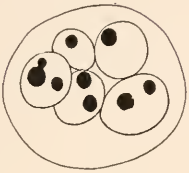

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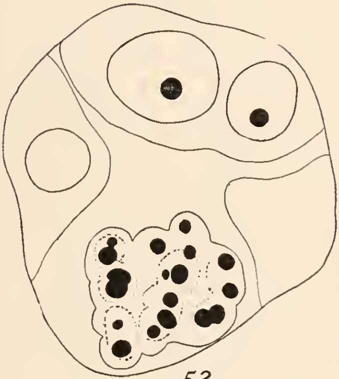

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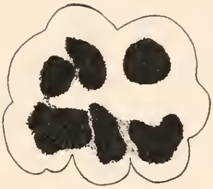

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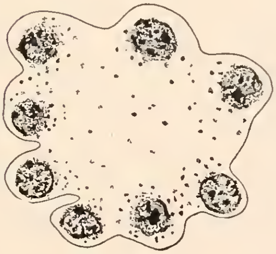

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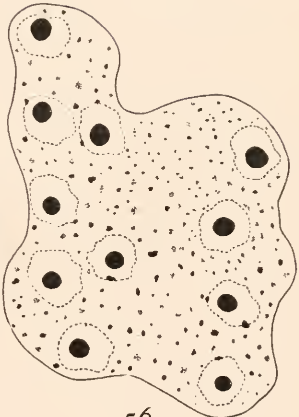

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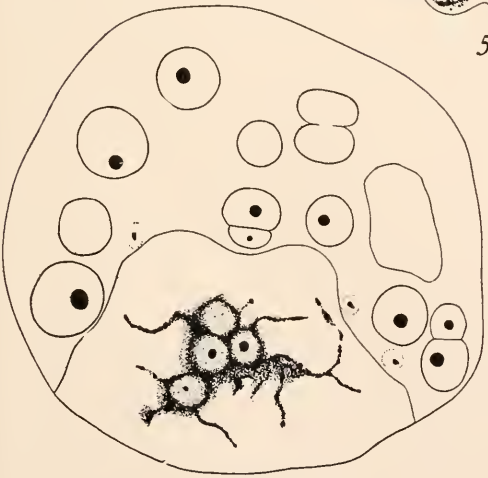

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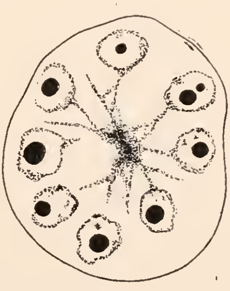

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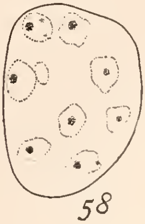

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PLATE XVI.

FIGS. 59, 60. One form of degeneration of cytophores after development of spermatozoa.

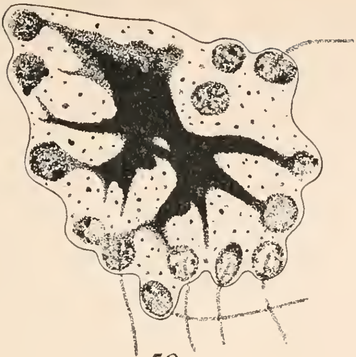
FIG. 61. Late stage of degenerating cytophore.

FIGS. 62, *A*, *B*; 63, *A–D*. Spermatogonia dividing amitotically; from full grown testes.

FIG. 64. Half of full grown testis; scale one half that of other figures; *a*, spermatogonia; *b*, early stages of spireme formation; *c*, *c*, spireme stage; *d*, preparation for first spermatocytic mitosis; *e*, dyads before first spermatocytic mitosis; *f*, *f*, cytophores with spermatids; *g*, cytophore with degenerating nuclei and spermatozoa; *h*, free spermatozoa; *k*, *k*, degenerating cytophores; *l*, shreds of protoplasm from earlier cytophores which have undergone degeneration.

The following figures are from *Moniezia expansa*: 1 *B*, 1 *D*, 3 *A*, 3 *B*, 3 *C*, 4 *B*, 4 *C*, 9 *D*, 10 *A*, 11 *A*, 11 *B*, 12 *A*, 13 *A*, 13 *B*, 14 *A*, 14 *B*, 15, 17, 18, 20, 21, 23, 26, 27 *D*, 28, 29 *E*, 30 *A*, 30 *C*, 31, 32, 34, 35, 36, 37, 38, 39, 40, 42, 44, 45, 46 47 *A*, 47 *B*, 47 *D*, 48 *A*, 48 *B*, 48 *C*, 49, 51, 55, 57, 58, 59, 60, 61, 62 *A*, 62 *B*, 63 *A*, 63 *B*, 63 *C*, 63 *D*, 64.

The others are from *M. planissima*.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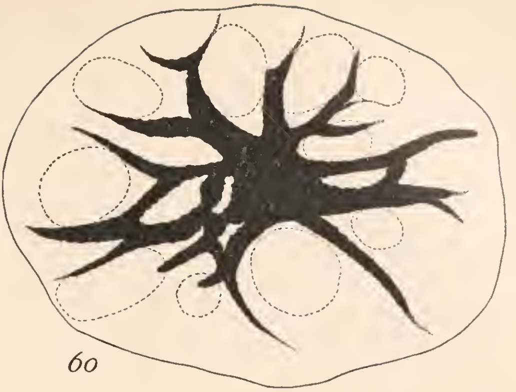
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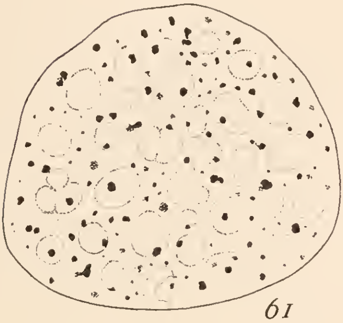
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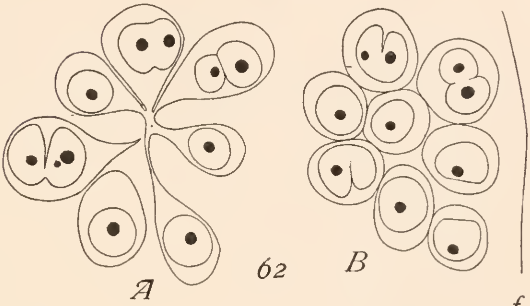

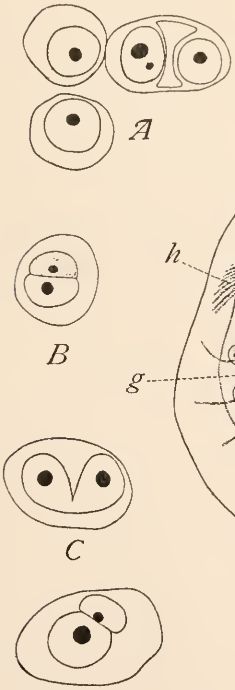

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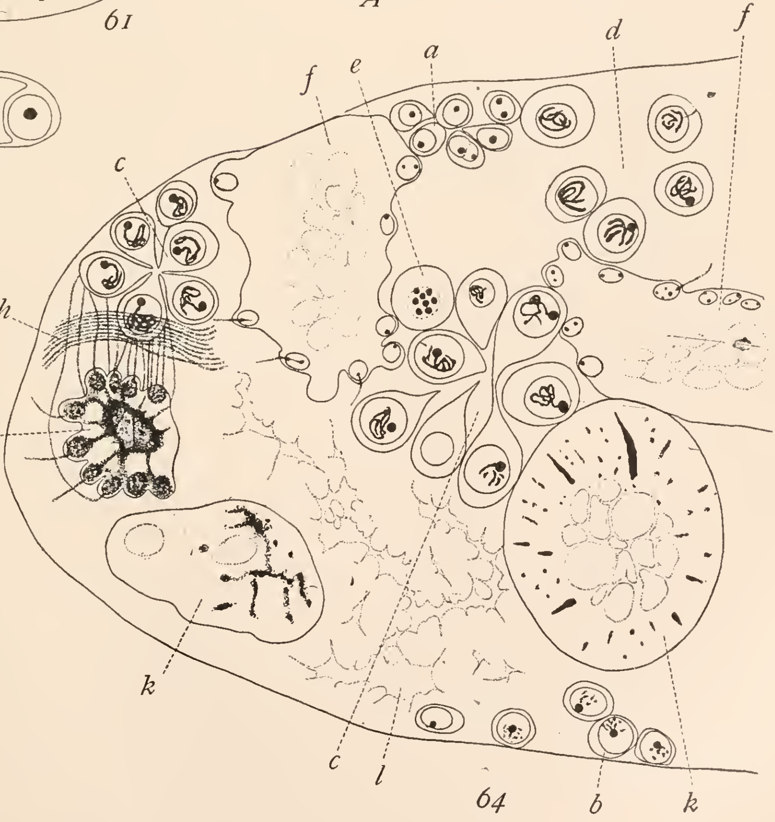
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THE CIRCULATORY AND NERVOUS SYSTEMS OF THE GIANT SCALLOP (PECTEN TENUICOSTATUS, MIGHELS), WITH REMARKS ON THE POSSIBLE ANCESTRY OF THE LAMELLIBRANCHIATA, AND ON A METHOD FOR MAKING SERIES OF ANATOMICAL DRAWINGS.

GILMAN A. DREW.

In considering any system of organs it is essential that we should bear in mind the modifications of the possessor of the organs, that adapt it to its particular life.

Pecten is one of the ablest swimmers among lamellibranchs. The whole structure of the animal is modified for this purpose. The valves have become rounded in outline, flattened, and comparatively light. The anterior adductor muscle has been lost, and the posterior adductor muscle, which is very powerful, is situated near the middle of the body. The cartilage has become well developed, so the shell may be opened quickly when the muscle relaxes, and the hinge line is straight, so there may be no unnecessary strains in opening and in closing the shell. Each gill is attached by one lamella only, so water in the temporary cloacal chamber may be thrown out without injuring the gills, and the gills and margins of the mantle are provided with muscles to withdraw them from the margins of the shell when the shell is closed. Furthermore the margins of the mantle are provided with infolded ridges and with circular muscles so it is possible to direct the current of water which issues from the shell in the required direction.

To fit the animal to a life of such activity, and to enable it to live in the comparatively exposed positions that it inhabits, an abundance of sense organs, tactile and probably visual, have been developed. These are placed in the most exposed positions, where they may give warning to their possessor, and are

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[1] Free use has been made of both descriptions and figures published as No. 6 of the University of Maine Studies, under the title of "The Habits, Anatomy, and Embryology of the Giant Scallop (*Pecten tenuicostatus*, Mighels)."

accordingly borne along the margins of the lobes of the mantle.

It is not entirely certain what relationship *Pecten* bears to the usual form of lamellibranch as regards positions of parts. In lamellibranchs that are supplied with two practically equal adductor muscles, a line connecting the two adductors runs nearly lengthwise of the animal. In such a case the hinge line is more or less dorsal, one end is anterior, and the other posterior. When one of the muscles disappears, as is the case with *Pecten*, one of the landmarks disappears and it becomes more difficult to locate the direction of parts. Inasmuch as the hinge line is usually dorsal, it is very natural to look at the hinge line of this form as dorsal, and for matters of description it is convenient to so consider it. If, however, the position that the anterior adductor would have occupied, had it been retained, be considered, the position of the mouth, foot and heart indicate that it would have to be placed much nearer the hinge line than the present position of the posterior adductor muscle, the muscle that is retained. If this is the case, it becomes evident that the loss of the anterior adductor muscle has been accompanied by a general reduction of the anterior part of the body, so a large part of the body of *Pecten* is to be considered morphologically posterior. This supposition seems to be borne out by the nervous system, and the vascular system of the mantle, as well as by the extent and position of organs. In most forms the margin of each lobe of the mantle is supplied with a posterior and an anterior pallial nerve of approximately equal size. These nerves supply the muscles and sense organs of the margins, and, in many forms at least, unite with each other so they form a continuous connective between the cerebral and the visceral ganglia. In *Pecten*, not only is this the case, but the nerve in the margin of the mantle is joined at intervals for nearly its whole length by nerves from the visceral ganglion (Fig. 6). On the other hand, it is joined only in the region of the anterior ear by nerves from the cerebral ganglion. The visceral ganglia are the important ganglia of the animal, and both the cerebral and pedal ganglia are greatly reduced.

The blood is supplied to the mantle very largely by the posterior pallial arteries (Fig. 5). The anterior pallial arteries are comparatively small, and while they are connected with the posterior pallial arteries, the size and character of the vessels indicates that the junction is probably very near the anterior ear.

Considering everything, it seems likely that the longitudinal axis of the body could be morphologically represented by a line drawn from near the hinge extremity of the anterior ear to the middle of the adductor muscle, and that a very small portion of the scallop is anterior.

Circulatory System.

The animal is large enough to allow one to successfully inject the chief vessels with starch or gelatin injecting mass, and then by dissection and microscopic preparations to trace the distribution of the vessels of the different organs and to determine quite definitely the course taken by the blood in its circulation.

The heart is a typical, symmetrical lamellibranch heart, with two auricles and one ventricle (Figs. 1 and 3), the latter perforated by the intestine which enters it near one end and leaves it near the other end (Fig. 2). Dorsally the ventricle is prolonged somewhat, posterior to the intestine, where the morphologically anterior aorta is given off, and ventrally to a less extent it is prolonged anterior to the intestine, where the much smaller morphologically posterior aorta is given off. The walls of the ventricle are of about even thickness throughout their extent, and are quite smooth outside and inside. The auricles join the ventricle on each side near its middle, are somewhat triangular in shape, with the most acute angle receiving blood from the gills and mantle at a point dorsal to the adductor muscle and directly ventral to, but some distance from, the cartilage. The opening of each auricle into the ventricle is near the middle of the side of the auricle that lies next to the ventricle and farthest away from the opening where the auricle receives its blood. The muscles around the openings of the auricles into the ventricle, and to a less extent around the openings through which the auricles receive blood, are well developed and must act as sphincters that tend to keep the blood from being regurgitated The walls of the auricles, unlike those of the ventricles, are roughened by pits that open into the cavities of the auricles.

Both auricles and ventricle are composed of interlacing muscle fibers, and are capable of great extension. In preserved specimens, the heart is usually contracted and is not very conspicuous. In such contracted hearts the cavities of auricles and ventricle are practically obliterated.

The heart lies in a somewhat triangular, spacious pericardial cavity that is dorsal to the posterior half of the adductor muscle, and ventral to the posterior portion of the liver. Posteriorly it is covered only by a somewhat thick, muscular membrane which separates it from the mantle chamber.

As already mentioned, two blood vessels leave the ventricle (Figs. 1 and 3), one from each end. Although they are not so placed in reference to the ways the terms are generally used in describing *Pecten*, the two ends correspond to the anterior and posterior ends of the ventricle in most forms of lamellibranchs. The posterior aorta is much the smaller of the two, leaves the heart ventral to the intestine (actually anterior to it) and divides immediately after leaving the heart, into two vessels, one of which, the smaller, follows along the intestine, supplying it and surrounding portions with blood. The other vessel turns almost at right angles upon leaving the aorta and enters the adductor muscle, where it divides into a system of vessels that supply the muscle with blood.

The anterior aorta is much larger than the posterior aorta, and supplies all of the remainder of the body. It leaves the ventricle dorsal to (actually posterior to) the intestine and very soon gives rise to a vessel which passes into and supplies the wall that separates the pericardial cavity from the mantle chamber. From the pericardium the anterior aorta follows along the postero-dorsal border of the liver to the base of the ear. Here it gives rise to the branch (Fig 3, *ppa*) which passes posteriorly to the extreme upper margin of the mantle that lines the ear, giving off along its course a number of branches, which supply this portion of the mantle. Here it divides into two vessels, a right and a left, each of which bends abruptly ventrally (Fig. 5, *ppa*) and follows along the margin of the respective mantle lobe about opposite the line of attachment of the infolded ridge of the mantle, alongside but external to the pallial nerve.

Very fine branches are given off from these vessels all along their courses, which further divide to form systems of capillary spaces that are finest and most numerous near the margins. Some of these capillary spaces are large enough to be injected with starch mass, and I have a preparation of the mantle lobe from which only the infolded ridge has been removed, that was dehydrated, cleared and mounted in balsam, in which the whole system of vessels can be traced. A gelatin mass not only fills the spaces mentioned, but passes out between the cells so that in sections it may be seen to be diffused throughout the tissue. This seems to hold good for all other parts of the body with the exception of the gills, in which organs the mass is more completely, but not entirely, confined to the blood spaces. The indication therefore is, that the blood spaces are not confined vessels, and that the blood functions as both blood and lymph. The posterior pallial vessel may be traced far anteriorly, gradually diminishing in size along its course. Here it finally joins the anterior pallial vessel. The anterior pallial artery (Fig. 3, *apa*) leaves the anterior aorta very near the cartilage and runs directly to the anterior border of the hinge region of the mantle, giving off vessels to this portion of the mantle on the way. Here it branches into right and left vessels, each of which bends abruptly ventrally (Fig. 5, *apa*) and pursues a course along the anterior border of the mantle similar to that taken by the posterior pallial artery at the other extremity of the animal.

Along the anterior border of the mantle, near the dorsal line, the vessel is rather small and slightly broken in its course. It may be possible that this represents the border line between the posterior and the anterior pallial arteries. There are other reasons for believing that a large share of the animal is morphologically equivalent to the posterior portions of other forms, and that the anterior portion is greatly reduced. This has received attention in another place.

Several vessels leave the anterior aorta to supply the liver and stomach. Most prominent among these is a vessel which leaves the aorta between the points of origin of the anterior and posterior pallial arteries. This bends out toward the left side of the liver, where, in injected specimens, it is very conspicuous, passes ventrally and sends branches to the major part of the liver and to the stomach.

A short distance in front of the cartilage the anterior aorta bends ventrally, passes through the liver and gives off a few small branches to it, sends a vessel to the palps in passing, and passes on to supply the foot and the visceral mass. The vessel that supplies the foot (Fig. 3, *fa*) leaves the aorta a short distance ventral to the mouth, passes along the body wall until the foot is reached, and extends into the foot along its dorsal border. Just before entering the foot this, the pedal artery, gives rise to a small vessel that passes posteriorly along the single retractor muscle of the foot, supplying it with blood. From the point of origin of the pedal artery the aorta extends into the visceral mass, following along the enlarged portion of the intestine that leads away from the stomach, and supplying this and other portions of the intestine and the reproductive organs with small and with large branches. The enlarged portion of the intestine that comes from the stomach is especially well supplied (compare Figs. 2 and 3), there being numerous small branches that are given off directly from the aorta, and large branches that follow along on the different sides of this portion of the intestine and likewise supply it with branches. A short distance ventral to the foot a large branch leaves the aorta and passes postero-ventrally to divide again and form small branches that supply the remaining loops of the intestine and the postero-ventral portions of the reproductive organs.

This completes what might be called the systemic arterial system. Beginning with the heart the system ends in the capillary spaces of the various organs. This system is most easily injected through the vessel in the suspensory membrane of the gills that is farthest from the adductor muscle (Fig. 1, *bv*), with a hypodermic syringe, injecting toward the heart. If a starch mass that will not pass through the capillary spaces is used, all of the vessels thus far described will be injected, as will also the veins that return blood from the gills, as this vessel is the one that returns blood from the gills to the heart. If a gelatin mass is used all of the systems may be injected, but as the injecting mass may pass out of the spaces, between the cells of the various organs, such injection does not aid in tracing the course of blood flow.

The systemic veins (Fig. 4) that collect the blood that is supplied by the systemic arteries, from the various organs of the body, may be injected from several different vessels. They may be injected by pushing the needle beneath the membrane that covers the posterior surface of the adductor muscle. A large blood space occupies this position, into which the needle is inserted and the mass injected fills the systemic veins. Another point from which these veins may be injected is from one of the superficial vessels of the visceral mass. These vessels are very conspicuous, and may be very easily picked up with the needle. Still another vessel is the vein that returns blood from the liver, which may be seen on the left side of the animal anterior to, but near the large artery that supplies the liver. Injecting any one of these vessels will to a greater or less extent inject the others, but there does not seem to be an entirely free communication between them. They all carry blood to the kidneys, and seem to empty into a common sinus on either side, that lies alongside the kidneys in the walls of the visceral mass. The sinuses of the two sides are connected beneath the adductor muscle, but it frequently happens that a complete injection of the system is not obtained from an injection from any one of the veins mentioned. Just where the obstruction lies in such cases has not been determined. It has been noticed that obstructions are more likely to be encountered in injecting from the veins of the visceral mass than in injecting from any of the others.

Inasmuch as blood spaces are cut in removing the muscle from the shell, it has been found desirable in injecting this system of vessels to wedge the valves open and to inject from the posterior surface of the adductor muscle. In injecting after the animal is removed, a considerable quantity of the injecting mass is sure to escape at the ends of the muscle.

The position of the veins may be seen in Fig. 4. A large vein comes from the liver, another from the foot, and the veins in the muscle unite to form a more or less definite sinus along the dorsal border of the muscle, and two smaller ones on the antero-ventral side of the muscle. These sinuses unite near the anterior ends of the kidneys. A series of vessels from the visceral mass unite along the borders of the kidneys and finally connect with these sinuses. Most of the blood from all of these organs is distributed to the kidneys through systems of capillary spaces. The branching of these vessels is not conspicuous on the surface of the kidneys, but is better seen by cutting the kidneys open. That not all of the blood necessarily traverses the capillary spaces of the kidneys is indicated by the fact that injections of the systemic veins frequently fill the veins that carry the blood away from the kidneys as well as those leading to it. This is much more frequently the case when injecting from the posterior surface of the adductor muscle than when injecting from other places, and seems to be dependent upon a direct connection between the vessel in question and the sinuses on the antero-ventral surface of the adductor muscle near the dorsal ends of the kidneys.

Of the blood that leaves the heart, only that which goes to the mantle remains to be accounted for. This is collected and returned directly to the heart (Fig. 5, *pv.*)

All of the blood that leaves the kidneys is conducted to the gills. The blood from each kidney is collected into a sinus that runs along the border of the kidney that is applied to the adductor muscle. This sinus, which also seems to receive blood from the sinuses on the anterior and ventral surfaces of the adductor muscle, bends abruptly ventrally over the anterior end of the kidney and is continued on the lower border of the suspensory membrane of the gill (Fig. 1, *ba*) to the posterior end of the gill, supplying the gill with branches throughout its length.

Blood vessels leave the vessel that carries blood from the kidney, opposite each of the inter-lamellar junctions of each of the gills supported by the suspensory membrane. Each of these branches is continued along the free border of the membrane that forms the inter-lamellar junction (Fig. 7, *ba′*) until it reaches the free edge of the lamella, the edge that is not attached to the suspensory membrane. That is, if the branch supplies an outer gill, it leaves the suspensory membrane along the free border of an inter-lamellar junction and crosses over to the free border of the outer lamella of this gill. Here the vessel is continued down the enlarged, modified filament that is concerned in the formation of the inter-lamellar junction (Fig. 7, *ba″*) giving out side branches through each of the inter-filamentar junctions (as long as these are composed of tissue that can carry blood vessels)[1] and so supplies the various filaments of the lamella. The blood thus distributed finds its way around the margin of the gill through small blood spaces and is continued up the other lamella of the gill, the blood of the small filaments being gradually collected through the vessels of the inter-filamentar junctions into the vessels of the large filaments (Fig. 7, *bv′*), and by these poured into a vessel that lies just beneath the vessel that supplies the gill and runs parallel with it (Fig. 1, *bv*). This vessel receives all of the blood from both of the gills of the side, and carries it directly to the corresponding auricle of the heart. Just before the vessel empties into the heart it receives a rather large vessel from the corresponding lobe of the mantle, which returns the blood that was sent to the mantle back of the heart.

To sum up the course of the circulation of the blood briefly, it will be seen that of the blood that leaves the heart only that which is sent to the mantle is returned to the heart after traversing a single set of capillary spaces; that a small portion of the blood sent to the adductor muscle (that which is collected by the sinuses on the antero-ventral portion of the muscle) may be returned after traversing two sets of capillaries — those of the adductor muscle and those of the gills; and that the greater portion is returned only after traversing three sets of capillaries — those of the general system, those of the kidneys, and those of the gills.

The reasons for this arrangement of the circulatory system are at least in part not hard to find. The blood which passes to the mantle loses some of its nourishing materials, but as the mantle lobes are thin and are bathed over such a large portion of their surfaces by a current of water, in which there is an abundance of dissolved oxygen, respiration, no doubt, takes place direct, and the blood has no need to pass through the gills to get a supply. Again the work of the mantle is not of such an active nature as to load the blood with nitrogenous wastes. It seems likely that the amount of nitrogenous waste in the blood that has traversed the mantle is so small that it would diminish the proportion of nitrogenous waste in the blood, if this blood were added to the blood that passes through the kidneys.

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[1] The inter-filamentar junctions near the free margins of the gills are composed of cilia only.

The blood that goes to the general system must in its progress lose a considerable portion of its oxygen, and in all portions except around the alimentary canal (where there is, of course, a decided gain) also food materials, and gain from the tissues a considerable amount of nitrogenous and carbonaceous wastes. It is then essential that such blood should go to the excretory and respiratory organs to get rid of these waste products and to gain oxygen. Inasmuch as the heart provides for but a single circulation it is necessary that the capillaries of these organs be traversed before the blood is returned to the heart. Why it is arranged so part of the blood may dodge the kidneys and be carried directly to the gills is not nearly so evident. Possibly the periodically great activity of the adductor muscle causes the blood to move through it so rapidly that the small kidneys cannot take care of it and properly perform their function, and the other channel is provided to carry the surplus away to the comparatively extensive gills where the increased flow can be taken care of with greater ease. It is, of course, essential that the amount of oxygen in the blood at such times shall not be reduced. It is at any rate evident that there is a possibility that part of the blood that is returned from the muscle, liver, etc., may not pass through the kidneys, for when starch injecting mass is injected through a vessel that carries blood from one of the kidneys to the gills, not only are the kidney and the gills injected. but part of the mass usually finds its way into the adductor muscle, liver, and other organs of the body.

The rate of the heart beat is slow, and as in other lamellibranchs is, no doubt, dependent upon the temperature of the animal as well as on other factors. The auricles and ventricle become very greatly distended during diastole, and contract so that their cavities are almost entirely obliterated in systole.

Nervous System.

The three pairs of ganglia that are usually found in lamellibranchs are present in this form, but they differ greatly in size and they are not all placed in the usual positions.

The cerebral ganglia (Fig. 6, *cg*) are placed some distance ventral to the mouth, just beneath the outer covering of the body. They, like the other ganglia, are yellowish in color, and may frequently be faintly seen through the covering of the body. Each cerebral ganglion is somewhat elliptical in outline with the long axis directed dorso-ventrally and has a rather distinct swelling on the ventral (actually anterior) and outer side (the side away from the median plane of the body) (Fig. 9, *cg*). The anterior end of each cerebral ganglion presents a forked appearance, due to the origin of two large nerve cords. The inner and ventral one of these two cords (Figs. 8 and 9, *cc*) is the commissure that joins the two cerebral ganglia. As the ganglia lie some distance ventral to the œsophagus, this commissure forms a long loop that passes dorsally around the œsophagus just posterior to the mouth. The outer and posterior of the two large cords that leave the anterior end of each ganglion is the anterior pallial nerve (Figs. 6, 8 and 9, *apn*). This runs parallel with the commissure as far as the œsophagus and is then continued along the side of the liver, and in the mantle, to the margin of the mantle in the region of the anterior ear of the shell, where it joins by several branches the circumpallial nerve (*cpn*) that follows along the margin of the mantle near the bases of the tentacles and eyes. The circumpallial nerve will receive attention later.

Between the points of origin of the cerebral commissure and the pallial nerve, a small nerve (Figs. 8 and 9, *pn*) leaves the ganglion to be continued dorsally, and to supply the labial palp.

From the inner, ventral surface of each cerebral ganglion, a little in front of the middle, the cerebro-pedal connective leaves to join the pedal ganglion of the same side. The cerebro-pedal connective is smaller near the cerebral than the pedal ganglion (Fig. 9, *cpc*) and bears a ganglionic swelling on its outer side very near the pedal ganglion.

In the acute angle formed by the surface of the cerebral ganglion with the cerebro-pedal connective, a small nerve (*otn*), the otocystic nerve, leaves the ganglion to be continued around the dorsal surface of the cerebro-pedal connective to the otocyst of the same side.

Posteriorly the cerebral ganglia taper rather gradually into the cerebro-visceral connectives, which run along the sides of the visceral mass very near the adductor muscle, until the visceral ganglia are reached.

The pedal ganglia lie very near each other (Fig. 9, *pg*), so the commissure that connects them is short and broad and presents ordinary ganglionic structure. They are separated from the cerebral ganglia only by a short interval, and lie anterior and slightly ventral to them, some distance dorsal to the base of the foot. They lie so near the surface that their color may frequently be distinguished through the body wall beneath the mouth. Two large nerves (*fn*) leave each pedal ganglion to be continued into the foot, where they supply the muscles of the foot and probably the byssal gland. The swellings on the cerebro-pedal connectives near the pedal ganglia have already been described. The otocystic nerves, which usually leave the cerebro-pedal connectives near the pedal ganglia, in this form originate directly from the cerebral ganglia near the point where the connectives leave the ganglia.

The visceral ganglia (Figs. 6, 8 and 10, *vg*) are by far the largest and most complicated of the ganglia, and from them nerves are sent to most parts of the body. They are situated on the antero-ventral surface of the adductor muscle, nearly opposite the external openings of the kidneys. They are imbedded in a mass of connective tissue and are fused to each other, so the commissure that connects them is nearly as broad as the ganglia themselves and shows ganglionic structure. The chief indication of the presence of a pair of ganglia is the arrangement of the nerves that leave them, and of the cerebro-visceral connectives that join them. The ganglia are divided into very definite regions, each of which is connected with definite bundles of nerve fibers and, no doubt, has a particular function to perform. I have not had time to make a detailed study of the structure and nerve tracts of the ganglia, but I am satisfied that there is much more complexity than is ordinarily attributed to the ganglia of lamellibranchs. The dorsal surfaces of the ganglia are quite smooth, but when seen from the ventral surface (Fig. 10) the regions that are indicated in the figure are always visible. On each cerebro-visceral connective, just before it joins the ganglion proper, there is a ganglionic swelling (*x*) that supplies one of two roots of a nerve (Figs. 6, 8, and 10, *bn*) that leaves in an antero-dorsal direction along the border of the excretory organ, to bend ventrally and posteriorly in the suspensory membrane of the gills, and supply the gills of the corresponding side. Between the points where the cerebro-visceral connectives join the visceral ganglia on the ventral side, there are four rather distinct swellings, with three less distinct swellings posterior to them. Extending laterally from the outer side of each ganglion is a somewhat flattened ridge (Fig. 10, *y*) from which all of the pallial nerves from this ganglion originate. These nerves (Figs. 6 and 8, *ppn*) pass laterally, posteriorly and anteriorly along the surface of the adductor muscle, to meet the mantle lobes and to be continued to the margins, where they unite with the circumpallial nerves. It will be noticed that they unite with the circumpallial nerve at intervals throughout the greater length of this nerve. As the pallial nerves that leave the visceral ganglia in most forms pass directly to the posterior portion of the mantle, the distribution in this form may be looked upon as evidence that all of this portion of the mantle belongs morphologically to the posterior portion of the animal.

Other nerves leave the dorsal surface of the visceral ganglia near their posterior ends, and enter the adductor muscle directly. The nerves that supply the posterior division of the muscle are continued along the ventral surface of the anterior portion of the adductor muscle until this posterior portion is reached. Small nerves also leave the ventral side of the ganglia and penetrate the visceral mass.

All of the ganglia are well supplied with nerve cells, there being very many large polar cells present, but the number of the cells is far greater and their arrangement more complicated in the visceral than in any of the other ganglia.

Nerve cells are also to be found in the circumpallial nerves and in the branchial nerves. So abundant are the nerve cells in the circumpallial nerves that they assume the structure of ganglia. The nerves by which they are connected with the visceral and cerebral ganglia contain no ganglionic cells. From the structural standpoint we would accordingly be justified in considering the circumpallial nerves as separate ganglia, and the nerves connecting them with the visceral and cerebral ganglia as connectives.

The circumpallial nerves of the two lobes of the mantle are connected with each other anteriorly and posteriorly near the hinge line (Fig. 8, *cpn*). They are not of constant diameter, but suddenly increase or diminish in size so that they have a rather irregular appearance. They lie just inside, that is, toward the median plane of the body, of the large pallial arteries that supply the mantle margin, about opposite the line of attachment of the infolded ridge. From them nerves are sent to the eyes and tentacles, to the infolded ridge and to the pallial muscles. Very likely the pallial muscles are partially supplied from the pallial nerves that come from the visceral ganglia, but of this I am not sure.

It seems probable that the ganglionic structure of these nerves has been developed to meet the needs of the very complex margins of the mantle. The development of ganglia in the immediate region of the sense organs is an indication of the ease with which such centers may be established when need arises.

The branchial nerves are supplied with ganglionic cells throughout their length. These are present not only along the borders of the gills, but from the points where the nerves originate to their extremities. The almost constant activity of the gills no doubt renders such an arrangement desirable. No other nerves or connectives in the body seem to be abundantly supplied with ganglion cells.

The whole nervous system is modified to meet the special needs of the animal. The cerebral and pedal ganglia are small, corresponding with the slight development of the anterior parts of the body and of the foot. The visceral ganglia are highly developed, corresponding to the excessive development of the parts that are supplied by these ganglia. Accessory centers have also been developed in the margins of the mantle and in the gills.

It seems that many students of Mollusca hold that the lamellibranch ganglia have been derived from a gastropod-like type, a type that possesses at least one pair of ganglia, the pleural, that are not commonly found in lamellibranchs. This view seems to be based largely upon the acceptance of a hypothetical type for a primitive mollusk that seems to me to be a much better ancestor for the gastropods than for the other classes of the Mollusca.

PHYLOGENY.

The hypothetical primitive mollusk that has persistently been offered for our consideration, and has found its way into a number of text-books, among which is Lang's "Text-Book of Comparative Anatomy," has the dorsal portion of the body covered by a conical shell, the foot flattened and adapted for creeping, a head fold that may be protruded from beneath the shell, a pair of plumose gills, and a nervous system with at least four pairs of definite ganglia, cerebral, pleural, pedal and visceral. *Distinctly gastropod throughout.*

If the development of animals is to be considered of any importance in pointing their possible lines of descent, and as long as embryo chicks have gill arches our belief has good foundation, it would seem that in those mollusks whose eggs are not loaded with yolk, whose embryos are not modified for protection in brood pouches, and do not have long larval histories that call for special modifications to enable them to cope with enemies and to get food, the embryos might be suggestive.

The presence of unlimited food and protection always tend to destroy characters. Thus we find that parasitic forms may have entirely lost organs that must have been well developed before the animals took to parasitic lives. The presence of a quantity of yolk furthermore frequently must have mechanical effects on the developing embryo that cause direct modification. Again those embryos that pass through long larval histories exposed to the competition of forms that would eat their food and other forms that would eat them, must necessarily be exposed to the same evolutionary factors, whatever they may be, that adult animals are exposed to and we would accordingly expect adaptive modifications in them.

There are many lamellibranchs, and not a few gastropods, that do not seem to be seriously modified by any of these factors and when their embryos are examined every one must be struck with their close resemblances. These embryos would seem to point to a free swimming ancestral form that obtained its food by means of surface cilia.

The first living forms that made their appearance on the earth must have used non-living substances for food. What the nature of these substances were, whether they were of a comparatively simple nature, like those that are used by our green plants to-day, or whether they were of an entirely different nature, we have no means of knowing, but it is evident that their food was not alive.

Then came the discovery by some form that the protoplasm of other forms could be used for food. This must have been the first great factor that led to the competition of forms and called for the improvement of bodily machinery among living things, to aid in the struggle thus begun, the struggle to get food and to escape from being used as food. As Professor Brooks has indicated,[1] this would naturally lead to the discovery and colonization of the bottom of the ocean because of the greater advantages it offered both for capturing food and in affording means of protection. This introduces the further element into the competition, of some positions being far more favorable than others, and as the struggle for position increased, a struggle that has never ceased, the competition, especially between close relatives, must have become very severe.

These factors, with the struggle dependent upon them, must have caused changes in structure (in the improved machinery that aids forms in getting food and in keeping from being used as food) to change very rapidly and it seems very plausible that in a comparatively short time in those days when forms were of simple structure and this keen competition was begun, the foundations of the great types of animal structure were laid.

We know that among our earliest fossils are to be found both lamellibranchs and gastropods, and it is back in the earlier time that we must look for the changes that have resulted in the formation of these classes.

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[1] Brooks, "The Origin of the Oldest Fossils and the Discovery of the Bottom of the Ocean," Smithsonian Report for 1894 (also Salpa).

We may possibly conceive that the ancestor of the Mollusca was among the early ones to recognize the advantages of the ocean bottom, and that its race soon developed a protective shell, if this had not started to form before it became a dweller on the bottom. The shell would offer protection, but would, because of weight, interfere with rapid movement. As enemies became able to get beneath its armor the shell became thickened and was made to cover the animal more completely, but the added weight interfered still more with rapid movement.

At this time we need not suppose that the animal had more than the very simplest nervous system, hardly more than that needed by a trocophore larva, for it would probably be dependent upon simple bands of cilia, or at the most a movable mouth portion, for getting its food. There is no reason for supposing that this animal had yet developed gills, or if gills were present they would hardly be more than simple folds of the mantle.

As competition became more severe, animals of this kind were in need of better protection, and it is possible to conceive that there might have been evolved two types, one that inclosed itself in a bivalve shell, crawled into the mud, and obtained its food by capturing the forms brought to it in a current of water of its own creation, the other, more like the *hypothetical primitive mollusk that has been described*, which retained a single shell and got its food by creeping over the bottom and picking it up directly. The first form would still have a simple head apparatus and would need new nervous centers only to provide for the mechanism necessary to crawl into the mud and the mechanisms necessary to create the current of water and capture the living forms from it. The second form would have a more complicated head apparatus and would need nervous centers to supply it and to supply the organ by means of which it was enabled to creep. In these differences in life, and in the consequent differences in structure, it seems reasonable to look for the differences in their nervous systems. If this conception is anything like true, from very early times there was no similarity in the method these two groups used in getting food. One has finally developed a remarkably satisfactory method of straining out living particles that serve it as food, from a current of water of its own formation, and is thus able to leave little of its surface exposed to the attacks of enemies. The other has developed one of the most complicated of machines in connection with its mouth to aid it in getting food.

As the head apparatus of the one type has increased in complexity, there has been greater need of ganglia to supply it, but in the whole line of development of the other type there has been no complicated head apparatus. About all of the actual evidence that we have of the presence of pleural ganglia in lamellibranchs is that given by Pelseneer,[1] who finds in *Nucula* and some other forms, that each anterior ganglionic mass is so shaped that it is possible to consider it as two ganglionic masses, and further that the connective that runs from this mass to the pedal ganglion is connected with this mass by two roots. The interpretation that he has put on this is that the two apparent divisions of the ganglion represent respectively the cerebral and pleural ganglion, and that the roots of the connective represent the cerebro-pedal and pleuro-pedal connectives that have become fused before reaching the pedal ganglion. My own view, discussed in another paper[2] is that the apparent division into two ganglionic masses is superficial, and due to the swellings accompanying the origins of nerves, and that one of the cerebral ends of the connective may be the central end of the otocystic nerve which is fused for the greater part of its length with the connective, but, unlike most forms, is free near the ganglion. This view seemed to me most reasonable as Stempell[3] has found that in *Soleyma togata*, a supposed near relative of *Nucula*, the otocystic nerve arises directly from the cerebral ganglion and is separate from the connective throughout its length. So far as I know, the instance given by Stempell is the only one that has heretofore been reported where the otocystic nerves originate from the cerebral ganglia, and are free from the cerebro-pedal connectives throughout their length. *Pecten tenuicostatus* has the same arrangement. In this form the position of the ganglia, connectives and otocysts is such that it is a very simple matter for the otocystic nerves to make direct connection with the cerebral ganglia, but they do not join the ganglia at their nearest points. Instead they are continued around the connectives to join the ganglia in contact with, and posterior to them.

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[1] Pelseneer, "Contribution á l'étude des Lamellibranchs," *Arch. de Biol.*, XI., 1891.

[2] Drew, "The Life-History of *Nucula delphinodonta*," *Quart. Jour. of Micro. Sci.*, Vol. 44, Part 3, New Series, 1901.

[3] Stempell, "Zur Anatomie von *Solemya togata*," *Zool. Jahrb.*, Bd. XIII., 1899.

To me it seems probable that the separation into the two groups that have developed into the classes Lamellibranchiata and Gastropoda took place at an early date in the history of the Mollusca, probably before a complicated head apparatus was developed, and while the nervous system was of a very simple nature. If this was the case, we have no reason to search for pleural ganglia in lamellibranchs, for it is very probable that they never had them. In fact were ganglia ever present in this region in lamellibranchs, it would be more reasonable to view them as new formations for special purposes than as direct descendants from, and accordingly homologous with, the pleural ganglia of gastropods. The gastropod and lamellibranch are so different in structure and habits that we may reasonably expect important differences in their nervous systems. Gastropods and cephalopods possess accessory ganglia that have evidently been developed to perform special functions. That such centers may be comparatively easily developed is indicated by the fact that the circumpallial nerves of the scallop are essentially such centers. Is it not then more likely that pleural ganglia have been developed in the groups that need them than that lamellibranchs, which, so far as we know, have never been more complicated than they are to-day, should have formerly possessed these ganglia and have since quite uniformly lost them?

Anatomical Drawings.

It sometimes happens that in making a series of drawings intended to illustrate different organs of the same animal, considerable labor can be saved by using a combination of photograph and ink. The figures of the present paper illustrate this saving much better than is usually the case. To draw the margin of the mantle, with its large number of sense organs, requires both time and patience, and were it necessary to draw it for each of the figures where it necessarily occurs, one would be tempted to abandon it altogether.

It occurred to me, while engaged in drawing this margin, that possibly it could be photographed on a paper of a quality that would allow pen drawing and thus save redrawing it. After some trials a platinum paper was found that met the requirements but I was surprised to find how much blacker Higgins ink was than the blackest print I could make.

Evidently, however, any mark that would take at all in making a zinc etching would print the same color as the rest when being put through the press, so one of the poorest of these photographs was finished with Higgins ink and sent away to have a zinc etching made from it. The result was perfectly satisfactory. It will be seen that the margins on Figs. 1, 2, 3, 4, 6, 11, and 12 are all alike. The margin of Fig. 1 is the only one that was made with pen and ink. Fig. 11 is a print of a negative made from this margin before the rest of the animal was drawn. Taking a print similar to that shown in Fig. 11, with pen and ink there was drawn into it the organs shown in Fig. 12. Fig. 2, before the alimentary canal was added, was the figure from which the photograph resulting in Fig. 12 was taken. The margin of Fig. 12 is then a photograph of a photograph of an ink drawing. The original of Fig. 12 was then worked on to form Fig. 2 just as the original of Fig. 11 was worked into Fig. 1. Figs. 3, 4 and 6 are all worked onto prints similar to that shown in Fig. 12. In the original paper in which these figures were published a number of others were based on photographs in a similar way. The saving of time in the paper probably amounted to more than one half, and certainly may be of importance to others. I have no doubt that photographs may also be made the basis of brush work, but great care will be necessary in such cases in getting the proper printing value. While the figures accompanying this paper show no evidence that the photograph and the ink had different printing valves, they would have been very unsatisfactory had they been reproduced by some other processes.

Summary.

Circulatory System. — The large size of the animal makes it possible to inject the vascular system successfully. Blood from the mantle is returned immediately to the heart. Most of the blood from other portions is carried to the kidneys, from which it is carried to the gills, and then back to the heart. A portion may dodge the kidneys and go to the gills. Blood seems to act both as blood and lymph. (See pp. 227–234 and Figs. 3, 4, 5 and 7.)

Nervous System. — The cerebral and pedal ganglia are small and somewhat removed from their usual positions. The visceral ganglia are very large and complicated in structure. The circumpallial nerves and the branchial nerves have ganglion cells throughout their length. The otocystic nerves originate directly from the cerebral ganglia. (See pp. 234–239 and Figs. 6, 8, 9 and 10.)

Phylogeny. — Ontogeny and the probable conditions that have resulted in the complication of structure, both seem to indicate that the division of the Mollusca into lamellibranchs and gastropods, took place at an early time, before the ancestors had attained much complexity of structure.

There seems to be no reason for believing that lamellibranchs ever had more complicated head machinery than they have at the present time. If this is true they probably have never had need of more anterior ganglia than they now generally have. (See pp. 239–243.)

Anatomical Drawings. — A combination of photographs and drawings may sometimes save much time and tedious work. (See pp. 243 and 244.)

University of Maine,
Orono, Maine,
November 15, 1906.

PLATE XVII.

FIG. 1. Animal as seen from the left side with the left shell valve and mantle lobe removed and with a portion of the pericardial wall cut away. A few of the blood vessels are shown. Two thirds natural size.

FIG. 2. Animal as seen from the left side with the left shell valve and mantle lobe removed, with the alimentary canal shown. Two thirds natural size.

a, auricle; *ba*, branchial artery; *bv*, branchial vein; *c*, cartilage; *e*, excretory organ; *f*, foot; *fe*, free edge of the unattached lamella of the gill; *g*, gill; *i*, intestine; *lp*, labial palp; *m*, mantle; *pa*, posterior adductor muscle; *s*, stomach; *v*, ventricle; *vm*, visceral mass.

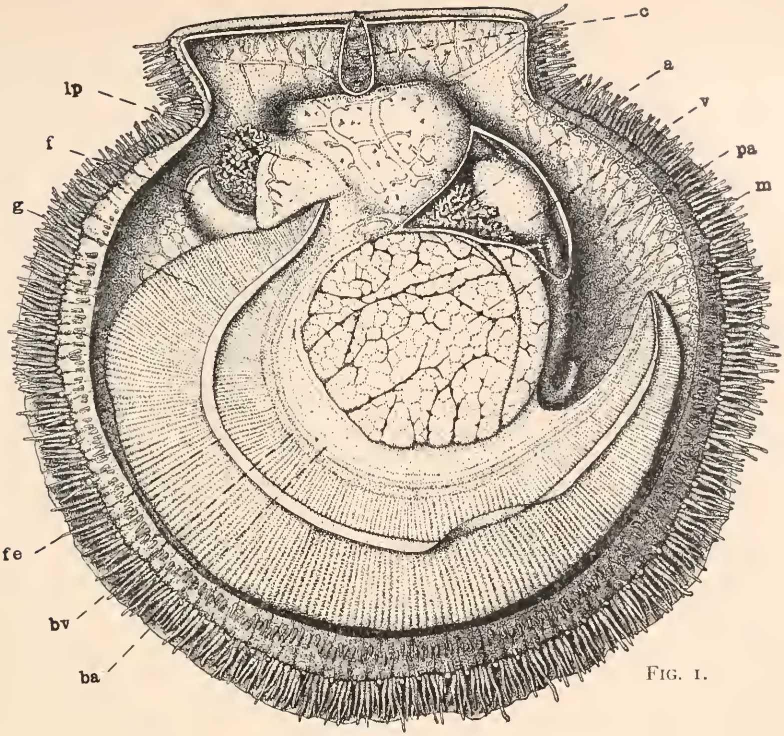
c
a
lp
v
pa
f
m
g
fe
bv
ba

FIG. 1.

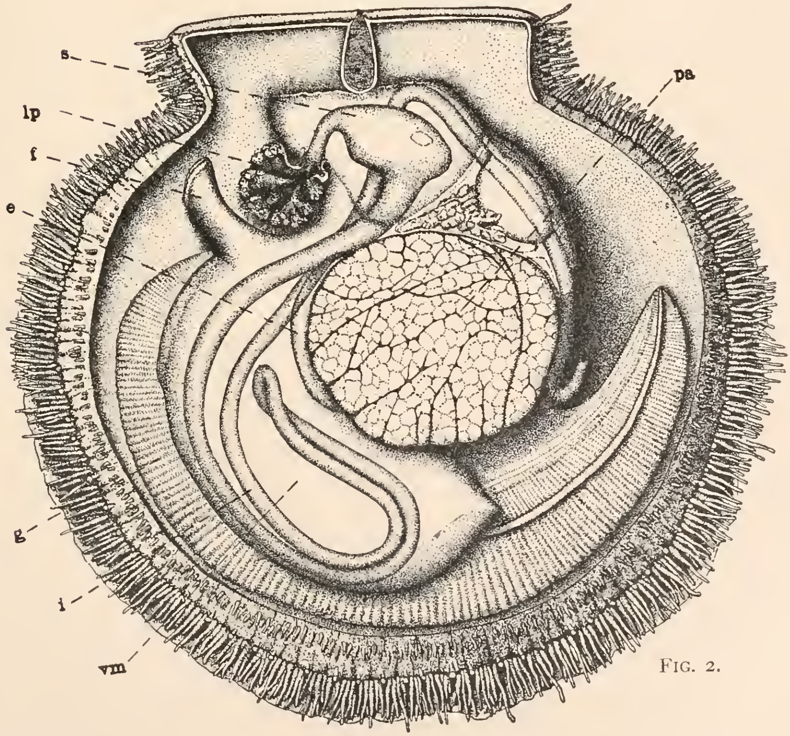
s
pa
lp
f
e
g
i
vm

FIG. 2.

Drew del.

PLATE XVIII.

FIG. 3. Animal as seen from the left side with the left shell valve and mantle lobe removed. Drawn to show the arterial system of blood vessels. Two thirds natural size.

FIG. 4. Animal as seen from the left side with the left shell valve and mantle lobe removed. Drawn to show the systemic veins. Two thirds natural size.

a, auricle; *aa*, anterior aorta; *apa*, anterior pallial artery; *e*, excretory organ; *fa*, foot artery; *fv*, foot vein; *ha*, hepatic artery; *hv*, hepatic vein; *pa*, posterior adductor muscle; *paa*, posterior adductor artery; *pav*, posterior adductor vein; *ppa*, posterior pallial artery; *v*, ventricle; *va*, visceral arteries; *vm*, visceral mass.

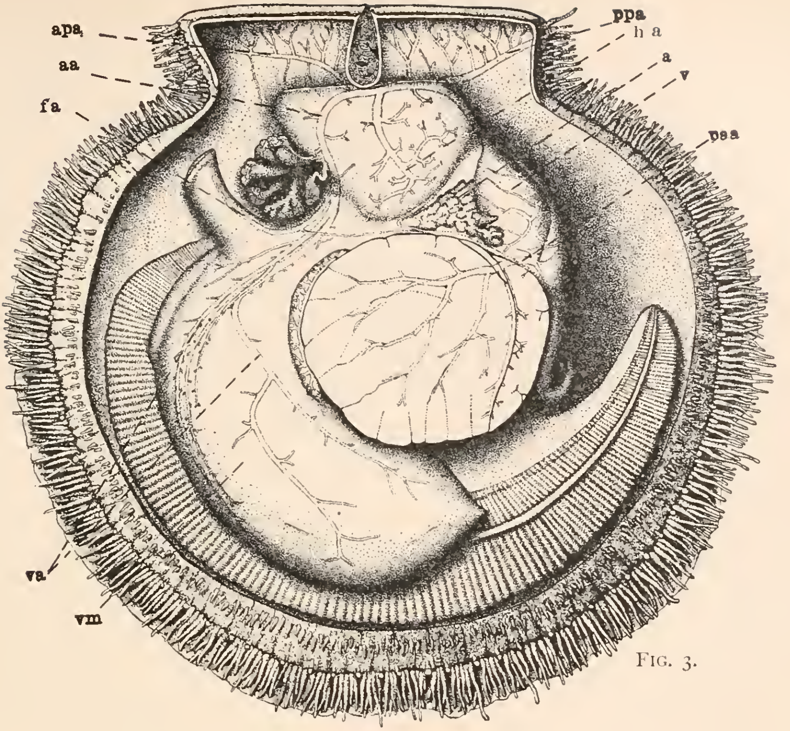
apa
aa
fa
va
vm
ppa
h a
a
v
psa

FIG. 3.

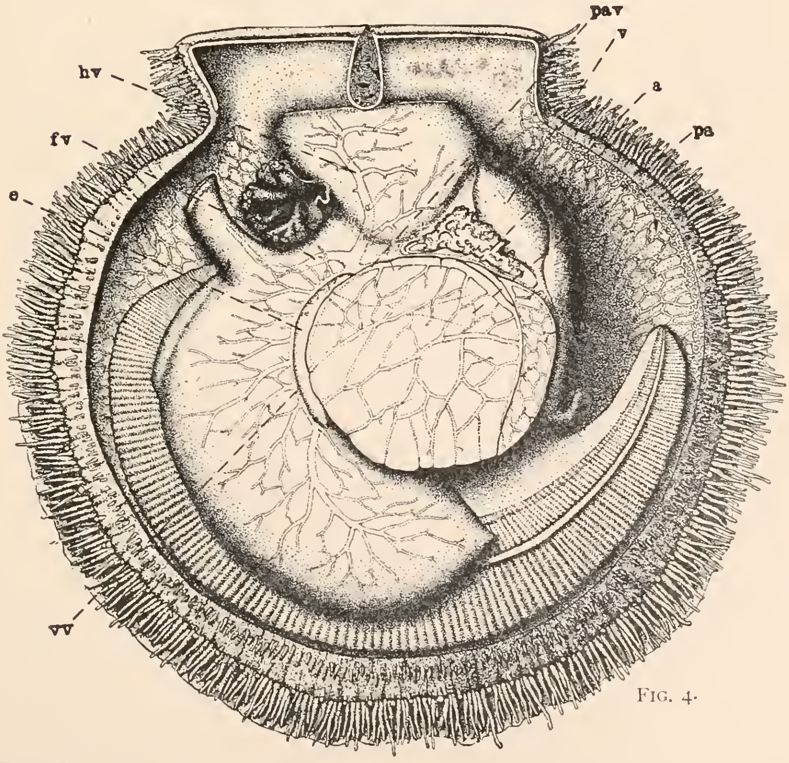
hv
fv
e
vv
pav
v
a
pa

FIG. 4.

Drew del.

PLATE XIX.

FIG. 5. Outer surface of the left lobe of the mantle showing the arrangement of blood vessels. Two thirds natural size.

FIG. 6. Animal as seen from the left side with the left shell valve and mantle lobe removed. Drawn to show the nervous system. Two thirds natural size.

apa, anterior pallial artery; *apn*, anterior pallial nerve; *bn*, branchial nerve; *cc*, cerebral commissure; *cg*, cerebral ganglion; *cpn*, circumpallial nerve; *cvc*, cerebro-visceral connective; *ot*, otocyst; *pa*, posterior adductor muscle (anterior portion); *pa′*, posterior adductor muscle (posterior portion); *pg*, pedal ganglion; *ppa*, posterior pallial artery; *ppn*, posterior pallial nerve; *pv*, pallial vein; *vg*, visceral ganglion.

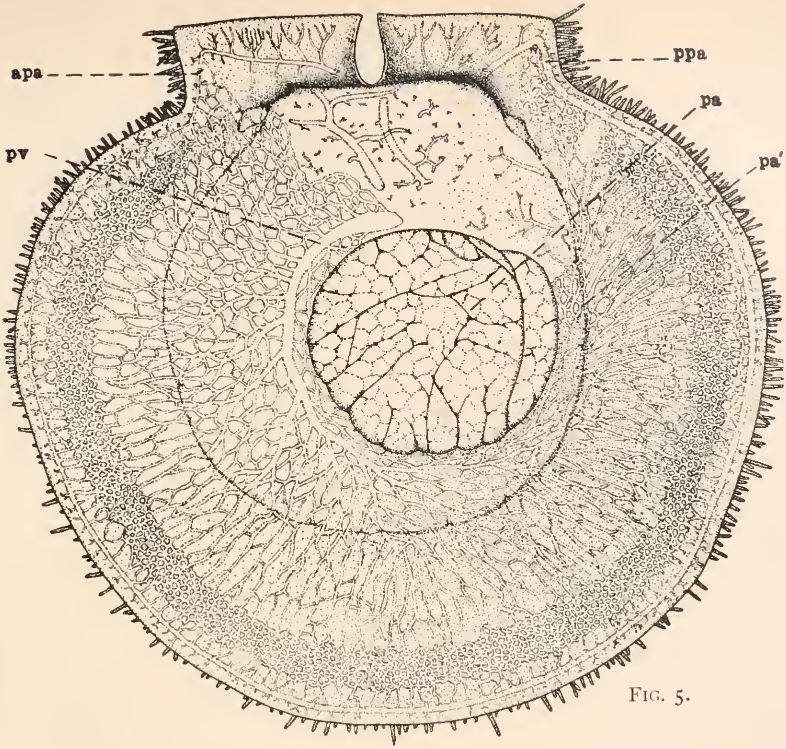
apa
ppa
pa
pv
pa'

FIG. 5.

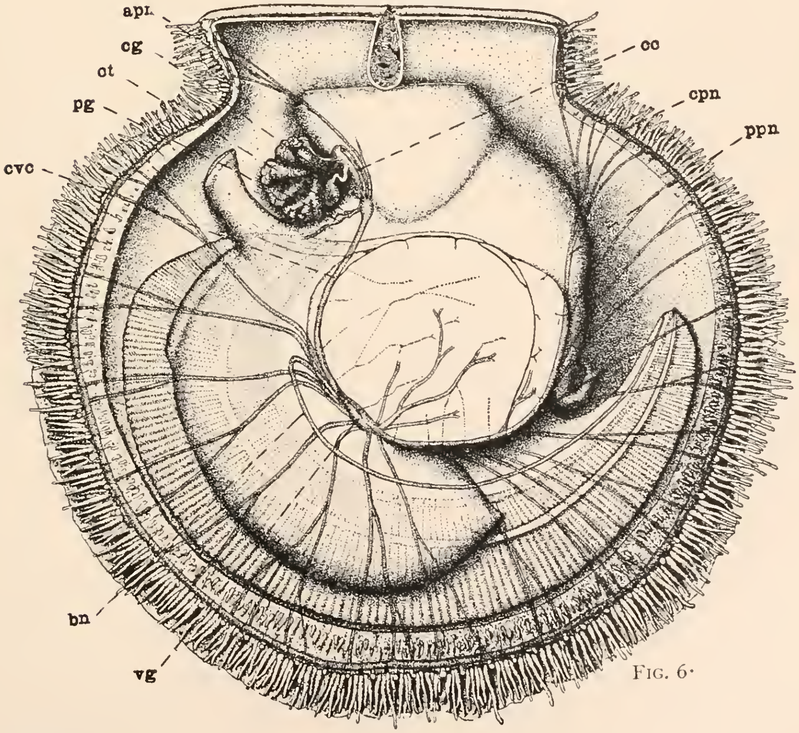
apn
cg
ot
pg
cvc
cc
cpn
ppn
bn
vg

FIG. 6.

Drew del.

PLATE XX.

FIG. 7. A portion of a gill showing the arrangement of parts. The figure indicates the inter-lamellar junctions cut at different levels. The further lamella is the one that was attached to the suspensory membrane and the vessel (*ba′*) was directly connected with the vessel that supplied the gill with blood (*ba*, Fig. 1). This vessel follows along the edge of the inter-lamellar junction to the free edge of the unattached lamella (the one on the side nearest the observer in the figure), where it bends back and passes down the modified filament as the vessel *ba′′*. Branches are given off from this vessel through the inter-filamentar junctions to supply the filaments. The vessel *bv′* is the vessel into which the blood that has traversed the gill is collected. It in turn communicates with the vein of the gill (*bv*, Fig. 1). Magnified about seventy diameters.

ba′, branch of the branchial artery; *ba′′*, branch of the branchial artery in the modified filament; *bv′*, branch of the branchial vein; *cr*, chitinous rod; *gf*, gill filament; *ifj*, inter-filamentar junction; *ilj*, inter-lamellar junction; *io*, inhalent ostium.

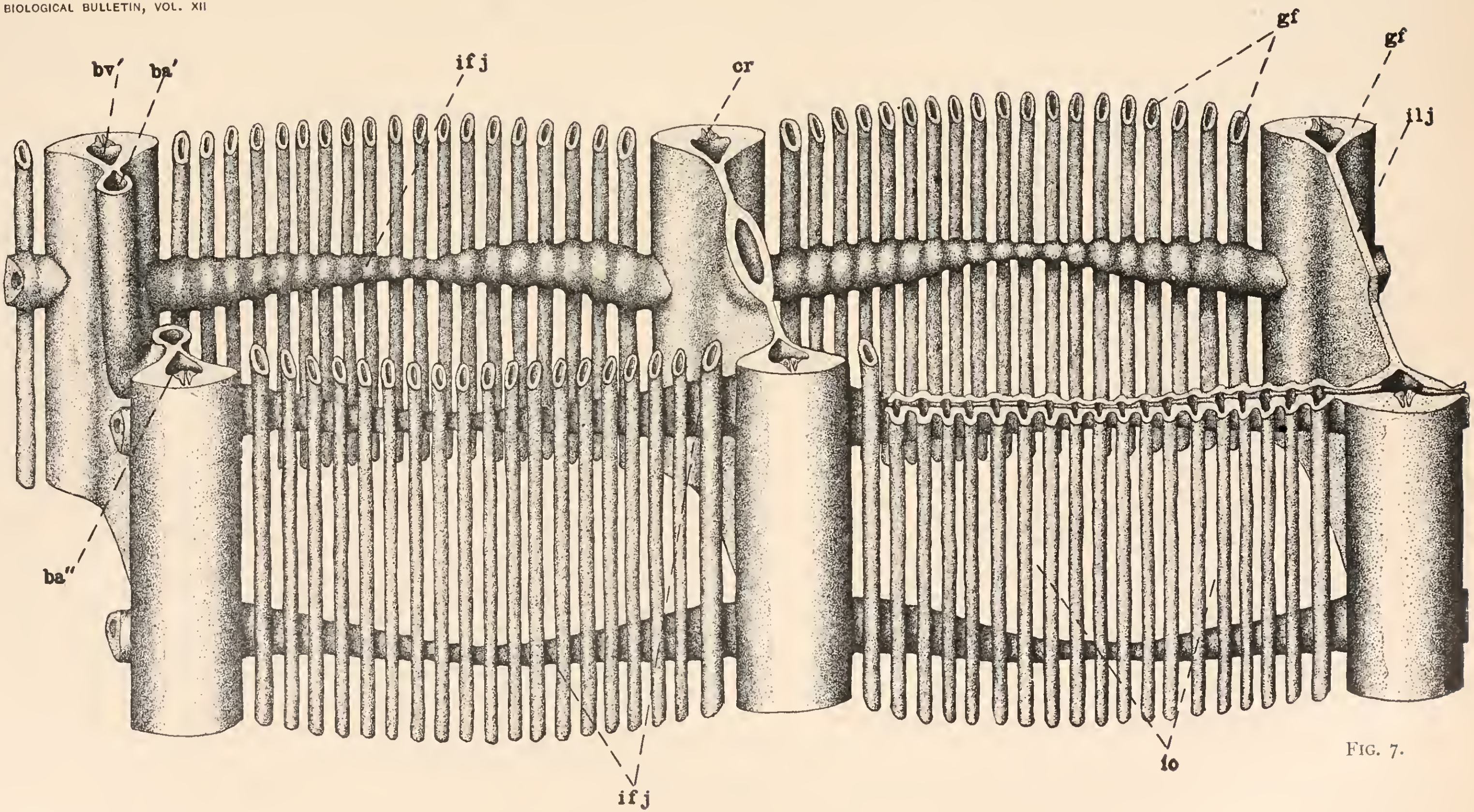
bv′
ba′
if j
cr
gf
gf
ilj
ba″
if j
fo

FIG. 7.

Drew del.

Plate XXI.

Fig. 23. Nervous system as seen from in front and a little to one side. Natural size. (Diagrammatic.)

apn, anterior pallial nerve; *bn*, branchial nerve; *cc*, cerebral commissure; *cg*, cerebral ganglion; *cpc*, cerebro-pedal connective; *cpn*, circumpallial nerve; *cvc*, cerebro-visceral connective; *ot*, otocyst; *pg*, pedal ganglion; *pn*, palp nerve; *ppn*, posterior pallial nerve; *vg*, visceral ganglion.

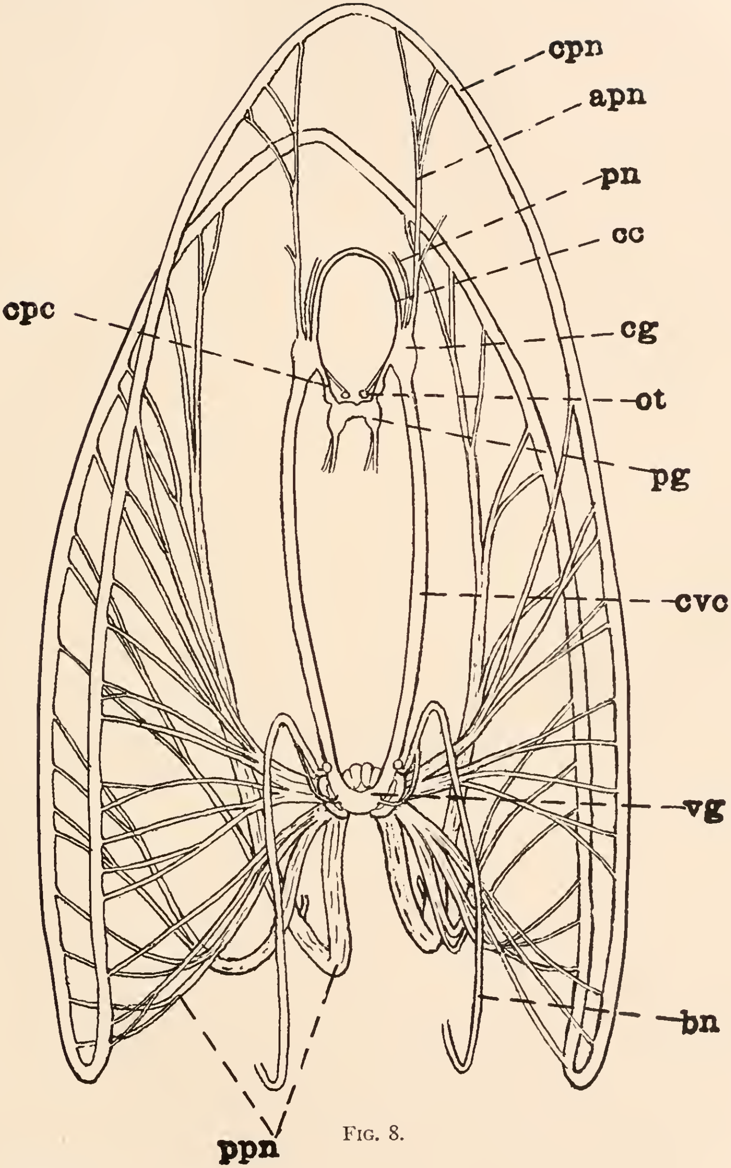
cpn
apn
pn
cc
cpc
cg
ot
pg
cvc
vg
bn
ppn

FIG. 8.

Drew del.

PLATE XXII.

Fig. 9. Cerebral and pedal ganglia with their nervous connections, as seen from the antero-ventral position. These ganglia and the otocysts lie in a mass of connective tissue and may be dissected out and mounted for study without injury Magnified about fifteen diameters.

Fig. 10. Visceral ganglia seen from the ventral side. These may easily be exposed for study by stripping the thin muscular covering from their ventral surfaces. They are hard to separate from the adductor muscle but they may be mounted with a thin piece of the muscle and studied in position. Magnified about fifteen diameters.

apn, anterior pallial nerve; *bn*, branchial nerve; *cc*, cerebral commissure; *cg*, cerebral ganglion; *cpc*, cerebro-pedal connective; *cvc*, cerebro-visceral connective; *fn*, foot nerve; *ot*, otocyst; *otc*, otocystic canal; *otn*, otocystic nerve; *pg*, pedal ganglion; *pn*, palp nerve; *ppn*, posterior pallial nerves; *x*, swelling on the visceral ganglion from which the anterior root of the branchial nerve originates; *y*, swelling on the visceral ganglion from which the posterior pallial nerves originate.

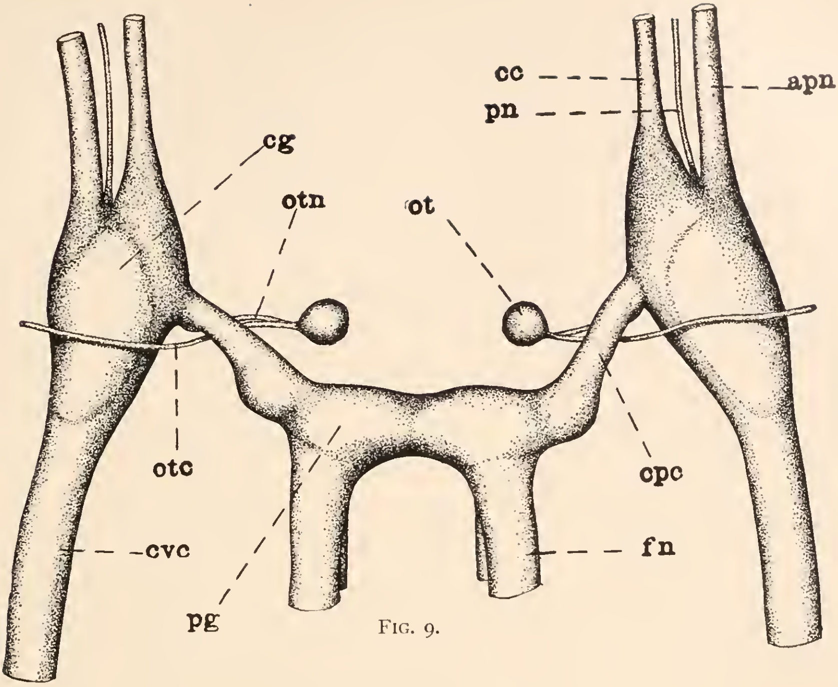
cc
apn
pn
cg
otn
ot
otc
cpc
cvc
fn
pg

FIG. 9.

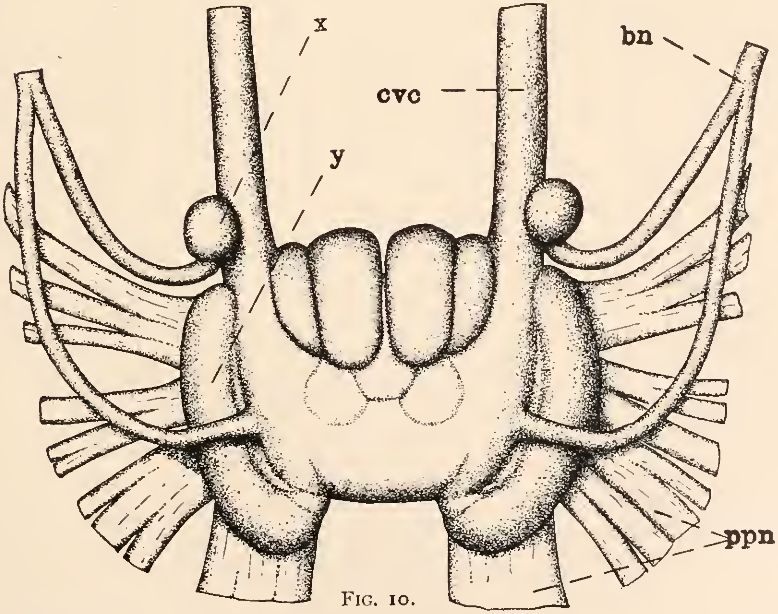
x
bn
cvc
y
ppn

FIG. 10.

Drew del.

PLATE XXIII.

Fig. 11. Etching made from a photograph of the margin of Fig. 1, before that figure had been completed. It will be noticed that the same margin occurs on all of the figures that show this portion of the mantle.

Fig. 12. Etching made from a photograph of a combination of a photograph and an ink drawing. The photograph was made from Fig. 2 before the alimentary canal had been worked in. Fig. 2 was drawn on a print like Fig. 11. Figs. 3, 4 and 6 are etchings of drawings made by adding various organs on prints like Fig. 12.

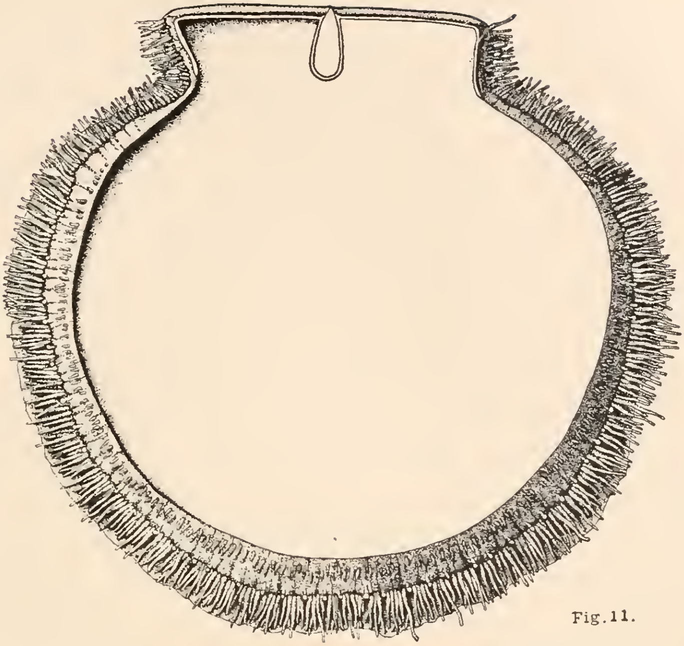

Fig. 11.

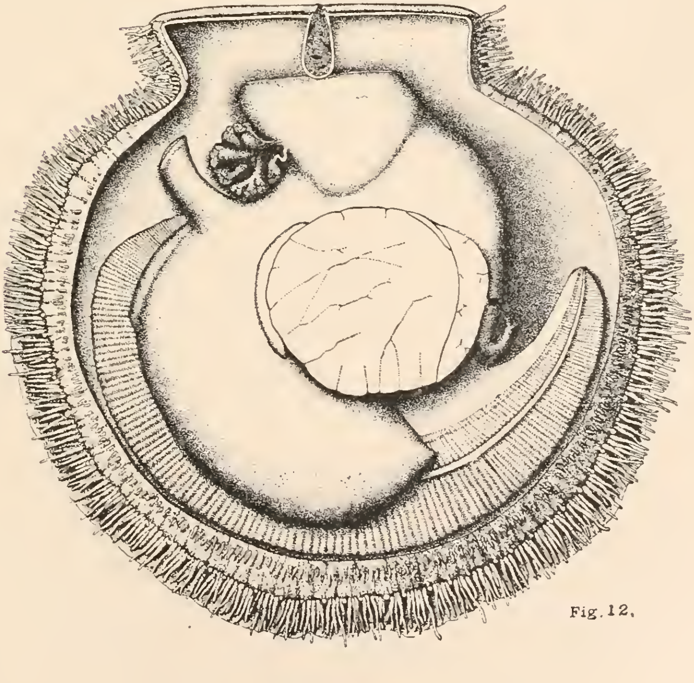

Fig. 12.

THE MATURATION OF THE MOUSE EGG.

WILLIAM B. KIRKHAM.

Sobotta ('95) after careful study of a very large number of preparations of the egg and ovary of the white mouse came to the conclusion that in nine tenths of these eggs the maturation processes involve the suppression of the first polar spindle, and the formation of only a single polar body. Gerlach ('06), after a study of preparations made at least as early as 1890, has revived Tafani's theory that in the majority of mouse eggs the second polar body is suppressed. Gerlach's conclusion is that when a spermatozoön enters an egg sometime after it has formed the second polar spindle, the second polar body fails to develop, and the spindle degenerates within the egg.

These results are at variance with the majority of opinions reached, before and since, by investigators of the eggs of other animals, vertebrate and invertebrate, and a reinvestigation of the maturation processes in the egg of the white mouse has brought it into line with most other metazoön eggs.

Material and Method. — The mice used have been killed during the period of most active breeding, namely, April, May, June and September, and serial sections made of the ovaries and Fallopian tubes. Ovulation, during the spring months, occurs very soon after parturition, independent of copulation, as observed by Rubaschkin ('05) in the guinea-pig.

When observed to be pregnant, the females were mated, and killed, some a few days or hours before parturition, others during that process, and still others at intervals from a few minutes to thirty hours after giving birth to a litter. The tissues were killed with a variety of the more generally used cytological fluids, and the following is a brief summary of the results obtained: All the ovaries contained some eggs with the second polar spindle and accompanied by the first polar body, and a majority of the series revealed ovarian eggs at the end of the spireme or with the first polar spindle. The eggs observed in the Fallopian tube fall into two main groups: those which had not been fertilized, and therefore retained the second polar spindle — some being accompanied by the first polar body, more without it — and those which had been fertilized. The latter included stages from the entrance of the spermatozoön through the cleavage stages.

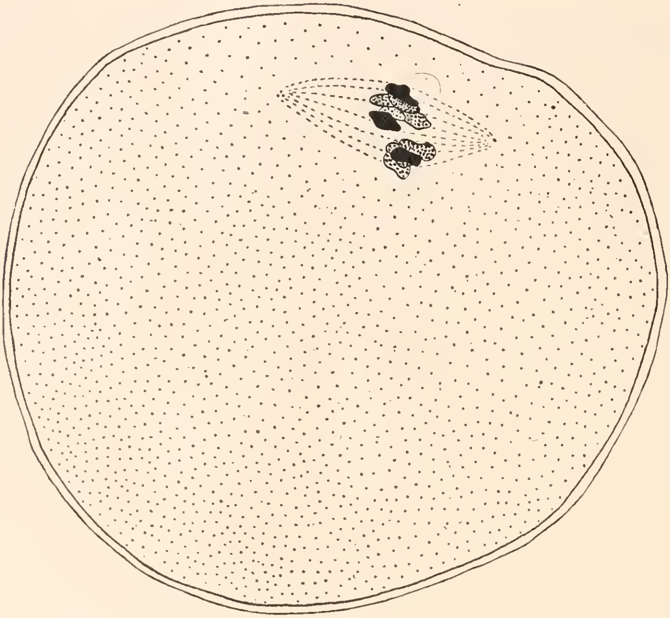

FIG. 1. Ovarian egg showing first polar spindle. Zona pellucida represented by double line. × 1200.

First Polar Spindle (Fig. 1). — The preparations in which there are stages immediately preceding the formation of the first polar spindle have not been fully studied, but there is evidence of a precocious division, the number of chromatin masses being between twelve and twenty-four.

The first polar spindle when first formed lies with its axis perpendicular to the radius of the egg, as found by Rubaschkin ('05) in the egg of the guinea-pig, and later one pole swings somewhat toward the center of the egg. The chromosomes of the first polar spindle are short and thick (Fig 2), and vary greatly in size. The spindle fibers come to more or less of a focus, and centrioles have often been seen at the poles of this spindle, where they are made up of several distinct, eccentrically placed granules.

First Polar Body (Figs. 3 and 5). — The study of many preparations reveals the following facts: None of the eggs in the Fallopian tube have failed to develop at least to the formation of the second polar spindle, and all the ovarian eggs which by their size, slightly denser protoplasm and large follicles appear to be nearly ripe, have already extruded the first polar body. The conclusion arrived at is, that apparently every egg which is capable of further development forms a first polar body within the ovary. This agrees with the observations of Rubaschkin ('05) upon the guinea-pig egg, and those of Van der Stricht ('01) upon the egg of a bat, *Vesperugo noctula*.

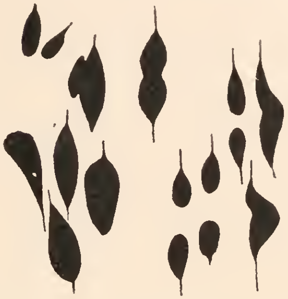

FIG. 2. Diagram of chromosomes in first polar spindle. Note great variation in size. Four more chromatin masses in adjacent sections.

This point established, it is next necessary to explain the disappearance of the first polar body in the majority of eggs seen in the Fallopian tube. The zona pellucida may persist in the mouse egg, undiminished, through the early cleavage stages, but in the majority of instances during the process of ovulation the first polar body is either forced through a weakened part of the zona, or frees itself by amœboid movements, and comes to lie outside the zona, as described and figured by Van der Stricht ('04).

The first polar body is usually oval in form, and is characterized, as found by Van der Stricht ('04) in the egg of *V. noctula*, by often possessing a little maturation spindle of its own, and in other instances having its chromosomes scattered. In some of these cases which possess a spindle, the first polar body would probably have divided mitotically, as observed by Sobotta ('95) in the mouse egg, and once by Rubaschkin ('05) in the egg of the guinea-pig. The polar bodies vary somewhat in size, and in one series of ovarian eggs there have been found first polar bodies of about four times the average volume. The number of chromosomes in the first polar body is twelve (dyads).

Second Polar Spindle (Fig. 3). — Immediately after the formation of the first polar body, the twelve dyads remaining in the egg are drawn into the equator of a new spindle, split longitudinally, and the twenty-four daughter, univalent chromosomes

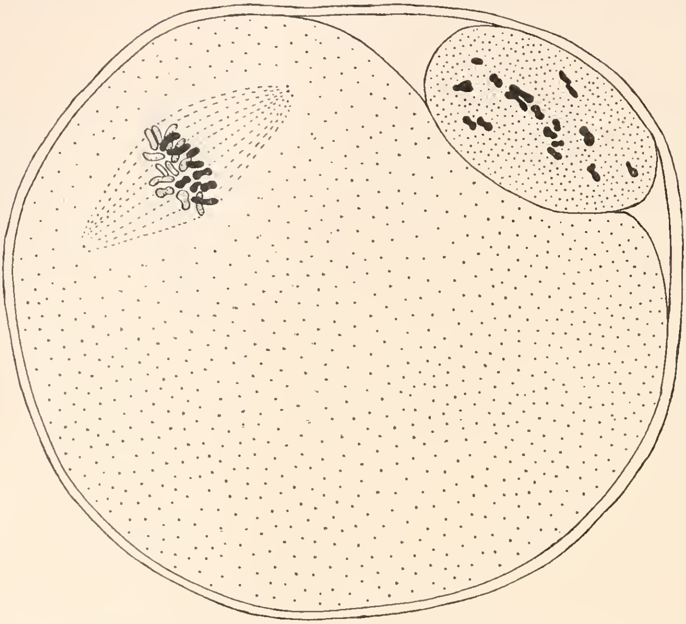

FIG. 3. Ovarian egg showing first polar body and second polar spindle. Seventeen masses of chromatin, some of which are undivided dyads, are scattered through the first polar body; twenty-four univalent chromosomes appear in the equator of the second polar spindle. Certain chromosomes have been added from adjacent sections. A minute centriole appears at each pole of the second spindle. The zona pellucida is represented by a double line. × 1200.

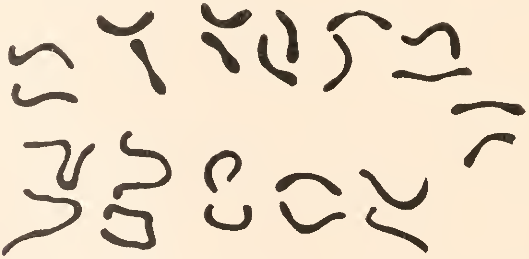

FIG. 4. Diagram of univalent chromosomes in second polar spindle, indicating difference in size.

lengthen out into filaments of various sizes (Fig. 4). Like the first polar spindle the second varies in size, and lies with its axis at right angles to the radius of the egg, usually near the first polar body. Centrioles, similar to those described above for the first polar spindle, have frequently been observed in second polar spindles, and in some cases a few radiating aster fibers have been seen at the poles. In attempting to determine whether a given polar spindle is first or second, the character of the chromatin has always been found a positive guide.

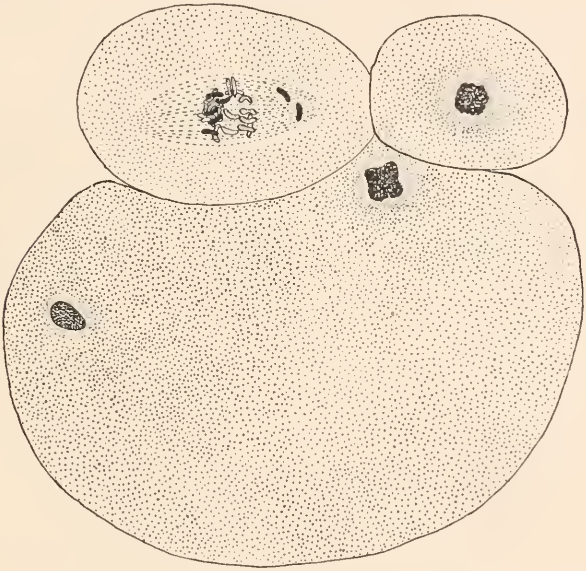

Fig. 5. Egg in Fallopian tube showing both polar bodies. Note spindle in first polar body. The sperm head appears at left, the female pronucleus at right, in the egg. × 1200.

Mature eggs which are retained within the ovary, together with such as are discharged and fail to be fertilized, degenerate with the second polar spindle, as found by Rubaschkin ('05) in the case of the guinea-pig egg.

Second Polar Body (Figs. 5 and 6). — Only one spermatozoön enters an egg, and it carries in most, if not all of its tail, a fact observed by Van der Stricht ('04) in the egg of *V. noctula*. When fertilized the egg at once forms its second polar body. This is more or less nearly spherical, smaller than the first polar body, and, as stated by Van der Stricht ('04) for *V. noctula*, generally has its chromosomes gathered into a single compact mass. It quickly forms a resting nucleus, possessing compact masses of chromatin, and is usually the only polar body seen during the early cleavage stages. In one instance (Fig. 6.) a second polar body was observed which had just been constricted off, and in consequence showed the separate chromosomes, twelve in number, and another preparation showed the second polar body forming the resting nucleus.

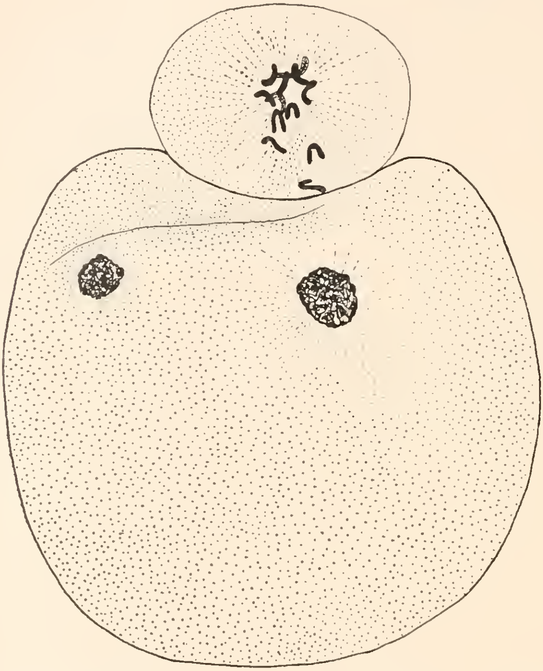

FIG. 6. Egg in Fallopian tube showing second polar body. First polar body has disappeared. At left in the egg is seen the sperm nucleus, and above it the separated tail of the spermatozoön; at right appears the egg nucleus, surrounded by delicate radiating fibers. × 1200.

The mouse egg is thus shown to be no exception to the general rule, that the maturation process in the metazoön egg involves the formation of two polar bodies.

In closing, I desire to express my gratitude, and great indebtedness to Professor Wesley R. Coe for his constant oversight and encouragement.

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ON THE ZOÖLOGICAL POSITION OF THE ALBINO RAT.[1]

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According to Leunis ('83) the black rat (*Mus rattus*) was known in Europe as early as the twelfth century, while the Encyclopedia Britannica (Olfield Thomas, '86) states the appearance of the black rat to be at least as early as the thirteenth century. Although the statements by the different writers as to the appearance of the black rat in Europe do not quite agree, yet it is clear that the arrival of the black rat was much earlier than that of the brown rat (*Mus norvegicus*)[2] which, according to various records, appeared in Europe at about the middle of the eighteenth century, or a little earlier.

Although both species of rats are described as originally natives of Central Asia, yet they are everywhere enemies. By the incessant competition between these two forms, the black rats were almost exterminated, first from Europe, and later from the greater part of North America, and at the end of the eighteenth century, the brown rats were alone found in abundance in these regions.

It is often stated that the white rat at present found in captivity, is the albino of *Mus rattus*. In support of this view there are a number of statements to be found in the older literature (Donndorff, 1792). (No effort has been made to examine the records previous to Linneus).

It is apparently on the basis of these records in the older literature that the current statements in popular natural histories and in encyclopedias are based.

On the other hand, in the zoölogical literature in the nineteenth century, there are numerous statements which refer to the albino rats as a variety of *Mus decumanus*.

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[1] From the Wistar Institute of Anatomy and Biology at Philadelphia.

[2] *Mus norvegicus*, Erxleben = *Mus decumanus* Pall. of older Zoölogical Literature. *Norvegicus* has priority, and has come into general use within the last two or three years.

Von Fischer ('69) in a catalogue of the mammals of the St. Petersburg Government, makes the following statement:

"Die Wanderratte, *Mus decumanus* Pall. (russisch Krýssa — Krýssa heist eigentlich *Mus rattus*, diese art ist bekannt unter dem namen Passjúck) kommt ueberall massenhaft vor in allen Farben; schwartz, schmutziggrau bis rostgelb, weissgescheckt und auch ganz weiss. "Die Hausratte, *Mus rattus* L., habe ich nie gefangen, weshalb ich annehmen zu durfen glaube, dass diesse Ratte hier auch nicht vorkommt."

Von Fischer ('74) used a white *Mus norvegicus* in his experiments on the production of hybrids. Later Crampe ('85) also used a white *Mus norvegicus* in experiments of the same nature.

Haacke ('95) and Bateson ('03) studied the crosses between the white *Mus norvegicus* and the common brown rat. None of the authors, however, describe in detail the white forms which they employed.

Despite the general belief to the contrary, there are many reports in recent literature indicating that groups of *Mus rattus* are still to be found in a number of localities, both in Europe and the United States.

In the United States, *Mus rattus* is reported from Texas, Florida and other southern states, and also from Iowa. Rhoads ('03) reports a number of new localities in the States of Pennsylvania and New Jersey. It has been learned through Director Dr. Seitz that in Germany the black rat is present in large numbers in the buildings connected with the zoölogical garden in Frankfurt a/m.

It may be interesting to note that the occurrence of white rats in a wild state has been reported from two localities in Iowa, by students working in the neurological laboratory at the University of Chicago. There are no means of determining, however, whether these were albinos of the black or brown rat. From this review it is evident, therefore, that there are, or have been, at least two forms of albino rats.

Since 1893 a colony of albino rats has been maintained in the neurological laboratory at the University of Chicago, and in 1906 a similar colony was established at the Wistar Institute of Anatomy at Philadelphia.

These colonies have been recruited for the most part from the northern states of the Atlantic seaboard, but some specimens have come from as far south as Missouri. All the rats received from these various localities have appeared to be of the same variety, and have always bred true.

Heretofore, the specific similarity of the albinos and the other forms has been concluded from observation of the external characters only. Wishing more exact information as to the zoölogical relation of the rats composing these colonies, the present investigation was undertaken to determine whether we were dealing with an albino variety of *Mus rattus* or *Mus decumanus.*

Externally, *Mus rattus* is usually distinguished from *Mus norvegicus* by the following specific characters:

Mus rattus is smaller in size. The tail of *Mus rattus* is considerably longer than the body, while in *Mus norvegicus* it is either shorter or only slightly longer than the body, but not relatively as long as that of *Mus rattus.*

The following measurements, though incomplete, serve to indicate this relation:

TABLE SHOWING LENGTH OF BOBY AND OF TAIL.

Observer.	*Mus rattus.*			*Mus norvegicus.*		
	Length Body.	Length Tail.	No. of Obser.	No. of Obser.	Length Tail.	Length Body.
New International Encycl..	21 cm.					27 cm.
Leunis	16 cm.	19 cm.			19 cm.	24 cm.
Hatai				27 males	21 cm.	24 cm.

The general shape of the head (see Fig. 1) of *Mus rattus* is slender, the nose is sharper, and the ear is both wider and longer than in *Mus norvegicus.* It may be worth while to mention that the so-called Alexandrian rat (*Mus alexandrinus*) is said to have external characters similar to those of the black rat (*Mus rattus*) and these two species are only distinguished by their coloring, *Mus alexandrinus* having a brown colored coat.

If we compare the external bodily characters of the albino rat found in our rat colonies, with those of the brown rat, we are surprised by their close similarity. All these characters of the brown rat are also characters of the albino rats composing our colonies. In other words, the common brown and our albino rats cannot be distinguished from one another by their external characters.

It is nevertheless true that the albino rats which we have examined, are smaller in size than the brown rats in the same localities. In fact, the absolute size of the albino rat is nearly intermediate between *Mus rattus* and *Mus norvegicus*. It is possible that the confinement in which these albinos have been reared, accounts for their smaller size, as the result of lack of exercise and altered conditions of life. It is possible also that we have here a phenomenon similar to that described by Semper ('81) and De Varigny ('94) on snails, where the size of the animals diminished with the size of the vessels in which they were reared.

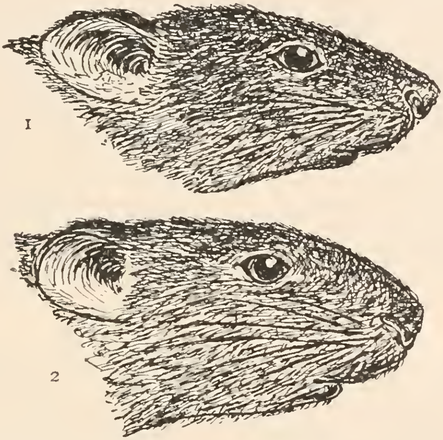

FIG. 1. Copied from "Encyclopedia Britannica," in order to show the shape of the heads of the brown and black rats. 1. *Mus rattus*. 2. *Mus norvegicus*.

It was thought that the character of the skull might serve for a more exact distinction of the forms under discussion. We therefore examined and compared the skulls of *Mus rattus*, *Mus norvegicus*, and of the albinos.[1]

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[1] In order to make this comparison, it was necessary to examine as many skulls as possible, and I am indebted to Professor J. A. Allen, American Museum of Natural History, at New York, Professor Elliot, Field Columbian Museum at Chicago, Dr. Greenman, The Wistar Institute of Anatomy at Philadelphia, and Professor Merriam, National Museum at Washington, for putting at my disposal various series of skulls, possessed by their several institutions.

To illustrate the differences found, both photographs and drawings have been made.

On comparing the skull of *Mus rattus* with the brown rat, the general unlikeness can be seen in Fig. 2.[1] The most noticeable difference is in the shape of the cranium.

When viewed from the dorsal aspect, the cranium of *Mus rattus* is oval in the outline, while that of *Mus norvegicus* is somewhat rectangular. Moreover, the dorsal aspect of the cranium in *Mus rattus* is decidedly convex, while in *Mus norvegicus* it is nearly flat. In *Mus rattus* the os nasale as compared to the entire length of the skull, is relatively shorter than *Mus norvegicus*. In *Mus rattus*, the outline of the os interparietale is somewhat semilunar in shape, while in *Mus decumanus* it is rectangular. In *Mus rattus*, the os parietale is broader as compared with its length, than in *Mus decumanus*. In *Mus rattus*, the foramen magnum is subcircular in outline, while in *Mus norvegicus* it is somewhat rectangular. On the ventral aspect of the skull, the large tympanic bullæ in *Mus rattus* are more conspicuous and eminent than in *Mus norvegicus*.

The junction point of the os basi-sphenoidale and os basi-occipitale is flat in *Mus rattus*, and protrudes in *Mus norvegicus*. The anterior end of the maxilla which forms the lateral wall of the infraorbital fissure, is blunter in *Mus rattus*, than in *Mus norvegicus*. The skulls of our albino rats are very similar in the above characters to those of *Mus norvegicus*, and the description of *Mus norvegicus* may be taken to apply to them.

In connection with the shape of the skulls, the determination of a cranial index has been made. The index used, was that obtained by dividing maximum width of the cranium by the length of the fronto-occipital line. (See Fig. 3.) On account of the small number of specimens measured, the accompanying table is to be considered as merely preliminary, but as it stands it shows a similarity in this index between *Mus norvegicus* and the albino rats, and a difference between these two forms and *Mus rattus*. The cranial index will be made the object of a more extended investigation.

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[1] Care has been taken to use only the skulls of fully matured animals. See J. A. Allen ('94) and H. C. Merriam ('95).

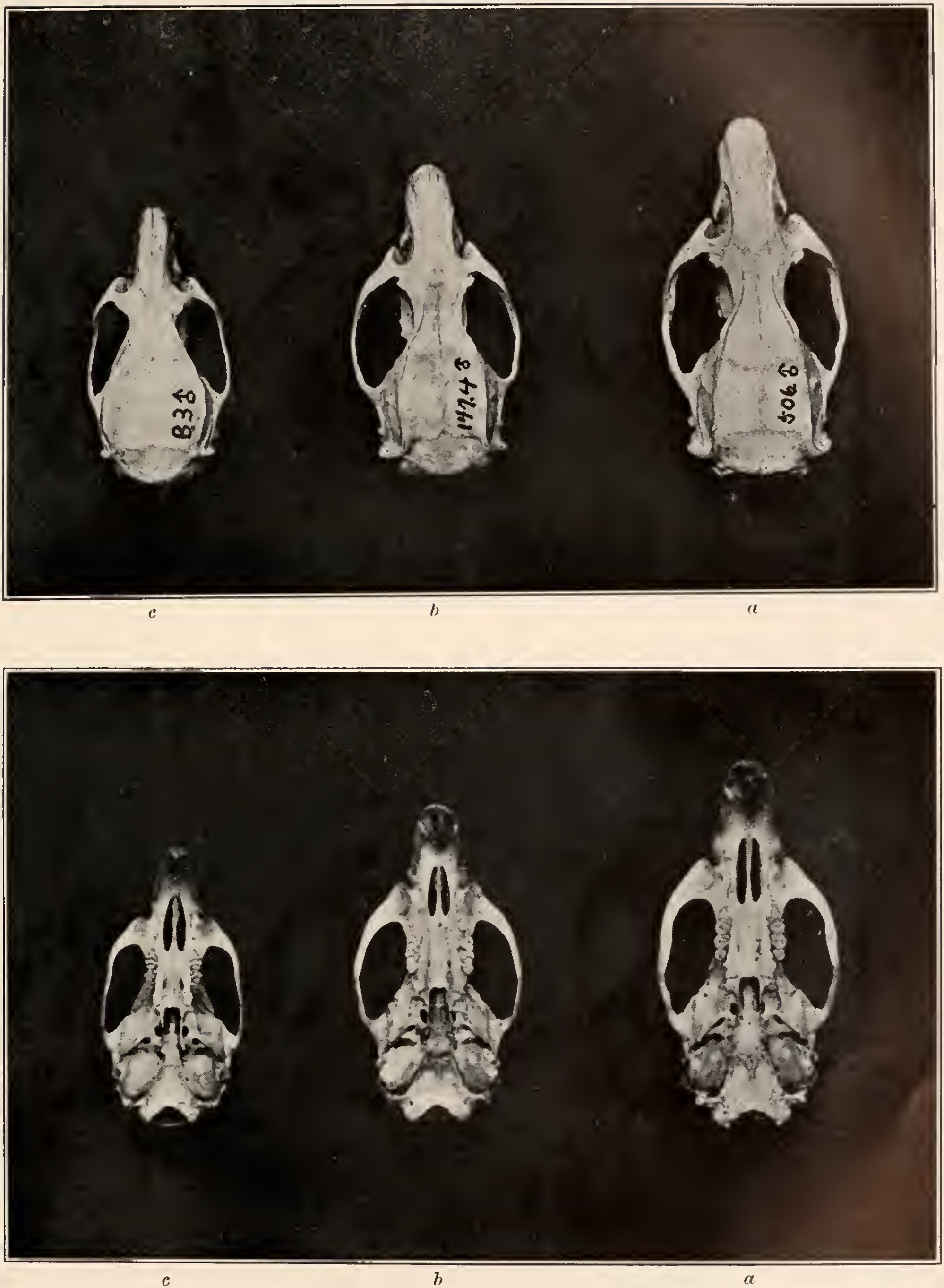

FIG. 2. Shows the skulls of *Mus norvegicus* (*a*), albino rat (*b*) and *Mus rattus* (*c*). The skulls were photographed from two different aspects, in order to show various views of the skulls for a comparison.

The upper row was taken from the dorsal aspect, and the lower from the ventral. The figures are about the natural size.

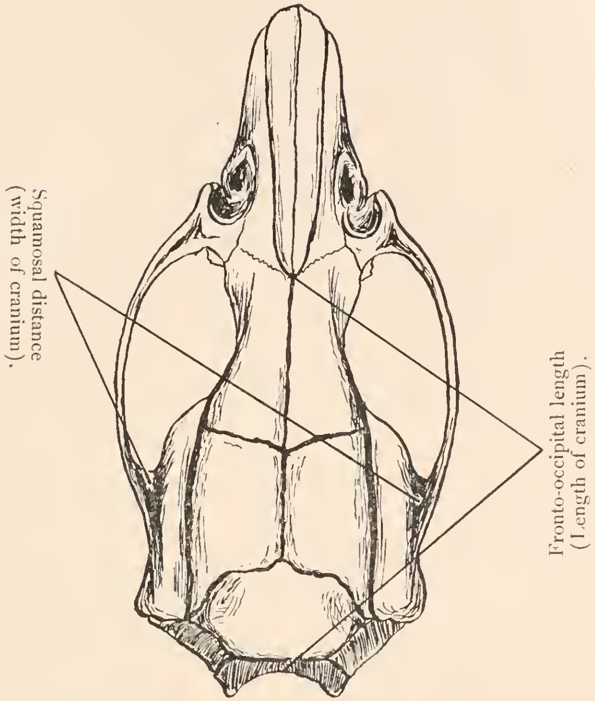

Fig. 3. (× two diameters.) The measurement of frontal-occipital length was determined in the following way:

Since the length measured from the tip of the nose to the posterior end of the inter-parietal bone, is not always equal to the length measured from the tip of the nose to the end of the occipital bone, both measurements were taken. First, the measurement from the tip of the nose to the end of the occipital bone, and second, that from the tip of the nose to the end of the inter-parietal bone. The difference thus obtained, was added to the length of the frontal-interparietal line, and the sum was called frontal-occipital length.

The width of the cranium was determined by taking a maximum width between the two points (right and left) where the zygomatic bones rest on the lateral walls of the cranium.

We conclude therefore, that the albino rats composing the colonies at Chicago and Philadelphia, are similar to *Mus norvegicus* in their bodily proportion, and in their cranial characters. They are however, smaller in size than the specimens of *Mus norvegicus* usually found.

TABLE SHOWING CRANIAL INDEX.

Males.	Cranial Index Average.	Extremes.	No. of Rats Used.
Mus rattus	.60	.58–.62	8
Mus norvegicus	.54	.51–.55	12
Albino rat	.54	.50–.56	12

Nevertheless this form is to be regarded as an albino variety of that species and to be designated *Mus norvegicus* var. *albus* (*oculis rubicundis*).

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NOTES ON THE BEHAVIOR OF SEA-ANEMONES.[1]

CHAS. W. HARGITT.

During the summer of 1901 while keeping a few sea-anemones in the aquarium for the purpose of studying their general habits, particularly those of feeding, my attention was drawn to the interesting phenomenon that certain species appeared more alert during the night, closing up more or less during the day. This was more noticeable in the large sand-anemone, *Eloactis producta*, whose peculiar habit of burrowing in the sand, enabled it to withdraw entirely when disturbed, or under other unfavorable conditions.

Having secured several specimens of this anemone they were placed in an aquarium, the bottom of which had been covered with sand to the depth of some six inches or more. The specimens, true to their habit, soon burrowed deeply in the sand, and lining the burrows with a slimy excretion they soon seemed quite at home. During the day they would be found with only the whorl of tentacles quietly protruding at the surface of the burrow, where their colors so closely conformed to that of the sand that the casual observer would hardly notice their presence. Going into the laboratory at night I was interested to see the specimens greatly extended, half of the body protruding beyond the burrows and tentacles raised in an attitude to seize passing prey. This was frequently observed afterward, and notes made of it at the time were recorded in which it was remarked that "these creatures are probably nocturnal in their habits."

At the same time I had under observation another anemone, *Sagartia leucolena*, a very common species about Woods Holl, and it was seen to migrate at times into darker portions of the aquarium, even creeping under bits of rock or other objects.

No further observations were made on the subject till the current summer. About a dozen specimens of *Eloactis* were collected and placed in the aquarium as before, and with the same result that such specimens as found sand proceeded to cover themselves as far as possible. In the light of current activity and interest in the matter of behavior it occurred to me to subject these creatures to a few experiments with a view of testing their reaction to light, and perhaps a few other environmental factors.

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[1] Contributions from the Zoölogical Laboratory, Syracuse University.

An examination of the available literature has brought to light but few instances in which any observations have been made concerning the behavior of actinians in relation to light. The Hertwig brothers, '79 ("Die Actinien Anatomisch und Histologisch," p. 191), cite brief observations made by Quatrefages on species of *Edwardsia* in 1842, and by Haime on *Cerianthus* in 1854, and include likewise brief references to their own observations on a deep sea-anemone, *Cladactis costæ*, in the Naples aquarium.

Quatrefages found that when a ray of light from a lamp was condensed upon the specimens by means of a lens they partially retracted. Haime observed that in bright sunlight species of *Cerianthus* contracted within their tubes and later expanded when the light became less intense. The Hertwigs record simply the fact the specimens during full daylight were more or less contracted and expanded as the light became less intense. "Im tageslicht zieht sie ihren Korper stark zusammen und erst wenn es zu dunkeln beginnt, dehnt sie sich auf das Vier- bis Funffache aus und entfaltet ihre Tentakeln, di zuvor eingezogen waren."

Jourdan has recorded a similar observation ("Les Sens chez Les Animaux Inferieurs," Paris, 1889), made upon a species of *Paractis* in which similar behavior was exhibited. "J'ai pu voir moi-même, sur des Actinies du genre Paractis, des manifestions evidentes de cette sensibilité spéciale. Des Orties de mer restent fermées aussi longtemps qu'on les expose à une lumière trop vive ; alles ne o'epanouissent que lorsqu'on les met à l'abri des rayons lumineux" (p. 221).

Eloactis producta. — My first observations were made to confirm those already cited, namely, to clearly demonstrate their nocturnal habit. Placed in the aquaria of the general laboratory, and in a few cases in smaller jars in my private laboratory, their behavior was closely watched after the specimens had become adjusted to their new habitat. In this connection should be mentioned the fact, to be discussed later, that some specimens were much less prompt in burrowing, a few remaining more or less indifferently upon the surface of the sand and showing but slight attempts to bury themselves.

It only required a few observations to determine beyond any doubt that only in light of low intensity, such as twilight, or in the aquarium under the rather dim light of an incandescent lamp at some distance, did the specimens protrude their oral portions and tentacles and show any degree of activity. To further demonstrate that these seemingly nocturnal activities were not merely a periodic response made at more or less definite intervals, the following experiment was made. A tall glass jar, some twenty inches in depth, the lower third of which was filled with sand, in which had been placed several anemones some two days previous, was so placed on a laboratory table that it was freely exposed to the diffused light of the room. Over the jar was placed about mid forenoon, when the creatures were securely withdrawn in the burrows, a blackened chamber or dark hood, so arranged as to exclude more or less perfectly the light. Removing the hood at the end of an hour it was found that the creatures were quite extended as at night. And it was soon evident, that with the removal of the hood and the admission of light, they were at once aware of the change and promptly began to show signs of irritation, which ended within five minutes in every specimen having retracted into its tube. To make certain that the response had not been induced by some mechanical stimulus, such as the tremor of passing steps, or an accidental disturbance of the table or the water in the jar, the experiment was repeated within a half hour and under conditions which made it possible to observe the phases of the response.

Within fifteen minutes after the chamber had been placed over the jar it became quite evident that the change had been recognized by the specimens. This was shown first by the extension of the tentacles, and next by a slow protrusion of the oral region by degrees, till within about half an hour the body was extended an inch beyond the surface, as before. Again removing the chamber and thus exposing the specimens to the light, within two minutes, indeed, almost immediately, they began to retract. This reaction is not sudden or general at once, as in such creatures as the earthworm, but begins in a somewhat indefinite movement of the body, accompanied by similar movements of the tentacles, followed very soon by a slow but definite retraction of the entire body within the tube, often including likewise the tentacles as well.

The experiment was later repeated in a room where it was possible to utilize direct sunlight. Under these conditions the reaction was much more energetic and definite, as might be expected. Variously modified, the experiments were performed repeatedly, perhaps fifty times, and with substantially the same results, though, as will be noted in a later connection, exhibiting variations of response. In some cases the reaction was so definite and prompt as to leave the impression on the observer that the creature was possessed of something akin to visual sensation. At other times the reactions were indefinite, sluggish, variable, and less convincing, though in the end resulting in the retraction of the specimen as before.

The following experiment was made to determine the extent of the sensory area, or in other words whether all portions of the body were similarly responsive to light. A specimen which had been quietly expanded on the surface of the sand for some time, being one of those which had shown less aptitude for burrowing, was so placed as to make it possible to reflect a narrow ray of light upon sharply defined parts of the body or tentacles. It was found that the oral region, including about one third of the body, was distinctly more sensitive than was any other. Light concentrated on the aboral portion seemed to have no effect at all, or so slight as to be indistinguishable. The tentacles were apparently less responsive than the immediately adjacent oral part of the body. This is slightly different from the condition found in *Sagartia modesta*, as will be noted later, and was a matter of some surprise, since the pigmentation of the tips of these organs might be thought to have some relation to sensory functions.

In a general way these results confirm the histological studies of the Hertwigs (*op. cit.*, p. 22), as to the distribution of the sense cells in actinians. They also agree substantially with some of their experimental observations as to the unequal distribution of the sensory areas, though on this point they gave slight attention to the effect of light as a stimulus (*ibid.*, p. 190).

Sagartia modesta. — This anemone has much in common with the former species. It is a creature having its habitat in the sand just below or near low tide line. Like the former it takes somewhat readily to the artificial environment of the aquarium, though seems somewhat less hardy under these conditions. I first studied this species in its native haunt, having found several specimens on an accessible beach. I first found them just before twilight, and in the shadow of a large boulder which still further reduced the light, with the tentacles extended very much as in the case of *Eloactis;* the body was not protruded beyond the tube. Going again in the brightness of early morning they were not to be seen, no sign of tentacles even in the partially closed burrow. I made these observations several times, and concluded that they were probably also nocturnal.

Specimens were collected and taken to the laboratory and placed in the same general conditions as were the former species. Experiments similar to the former were performed, but with much less promptness or clearness in reactions. Placed under the dark chamber there was not the ready extension of the body as in *Eloactis.* Further, on removal of the hood the response was much less sharp and convincing, though quite evident. Placed on a table upon which a beam of sunlight could be reflected it was found when the ray was reflected upon the numerous tentacles that there was immediate reaction. It should be stated that in this species the tentacles are very numerous, even a hundred or more, and form a dense crown in expansion covering the oral region like an umbrella, while in the former species these organs are but twenty in number and rather short. In *Segartia* the tentacles seemed more sensitive than in the former species, or than the oral region, but this may be due in some measure to their numbers, and to the general relations they sustain to the oral portion of the body, especially the region just below the tentacles. Still the results agree again with the views of the Hertwigs, as expressed in the following words: "Die Sinneszellen finden sich im Ektoderm der Mundscheibe und der Tentakeln, wie uns schien, überall ziemlich gleichmässig vor; nur an der Spitze der Tentakeln mochten sie vielleicht in grösserer Anzahl vorhanden sein" (*op. cit.*, p. 22).

Similar experiments were made on three other species of anemones, namely, *Sagartia leucolena*, *Sagartia luciæ*, and *Metridium marginatum*. These species are all more or less free, and variable as to habitat. The first, *S. leucolena*, is fairly common at various points along the shore-lines of the region of Vineyard Sound and southward. Its usual habitat seems to be under rocks near low tide, though taken also on the piles of docks. It seems to seek the under sides of rocks, or settles among masses of *Molgula*, sponges, etc., on piles, thus more or less secluded, and seldom seen by the casual observer.

On the other hand, *S. luciæ* seems to be equally at home almost anywhere in shallower pools, on fucus, piles of docks, etc., sometimes in shaded places, but oftener in the open sunlight on rocks, fucus, etc. About the same may be said of *Metridium*. While more common from deeper water than either of the others, it is yet quite common just below tide line on rocks, piles, etc.

The experiments on these species were made under the environment of the aquarium, but were sufficiently varied to give fairly satisfactory tests as to their reactions to this class of stimuli.

From what has been said as to the habitat just given it might be inferred that *Sagartia leucolena* would prove the more responsive to the tests, and such was found to be the case without exception, though as in the former cases, with considerable individual differences.

Verrill long ago pointed out that this species was more active when in dimly lighted aquaria, or at night. However, I have not found that specimens in the general light of the laboratory showed any very evident light reactions. But when an aquarium was placed in direct sunlight there was an almost uniform attempt on the part of specimens to escape from the direct rays. As a rule this was done by slowly creeping over the edge of the stone or shell into a less exposed position. Specimens which were in glass jars, and attached to the sides or bottoms of the jars, when brought into direct sunlight soon closed up entirely, withdrawing even the tentacles, and assuming a more or less hemispherical shape. Taken from the direct light into the diffused light of the room they promptly expanded and remained so until again placed in the sunlight. This experiment was repeated again and again, and with substantially the same results. It was also found that the degree of contraction was very closely an expression of the degree of light intensity.

Many specimens were brought to the laboratory adhering to small rock fragments, bits of shells, etc. In a few cases when such specimens were exposed to direct light they would creep over to the shaded side of the rock, and during the night return to apparently the exact spot previously occupied. This might be taken to suggest some such sense of position as is known to be had by certain gasteropods; but the tests were not sufficiently numerous nor constant to warrant any definite statement.

With *Sagartia luciæ* and *Metridium* the case was very different, as might be expected. Specimens of these anemones placed under the same conditions as the former, indeed in many cases when occupying the same aquarium, were found to be almost without exception, quite indifferent to light. Placed for some time under a dark hood and suddenly exposed to direct sunlight there was not the slightest evidence that there was any sense of the change. The experiment was made in various ways. Sometimes as just suggested. Again, a beam of strong light was reflected directly on the specimen as it was quietly expanded on the table, but so far as *S. luciæ* was concerned, always and without exception, with negative results. Occasionally, though always doubtfully, *Metridium* would show some slight sensory movements of the tentacles. But specimens have been subjected to the reflection of a strong beam of light directly upon the oral surface for ten minutes at a time without the slightest response.

I have had a few specimens of *Edwardsia elegans* in the aquarium but for some reasons they did not seem at ease under these conditions, and exhibited no distinct evidence of any photic sensibility. I have seen but once any living specimens of *Cerianthus* at Woods Holl and then only under circumstances which rendered any observations impracticable. I regret therefore, not to have been able to test the sensory behavior of these species.

The only other aspects of behavior which have been observed are those of feeding, and the very variable reactions concerned in tube-building.

Concerning the former my first experiments were made several years since. At that time I tested their feeding propensities by trying in various ways to induce them to take food. At various times during their aquarium life I tried to feed the creatures with bits of crab meat, bits of fish, clam, etc., but in no case was I able to induce the creatures to take the bait. During the present summer I observed that specimens of *Eloactis* which had been dug up and placed in a pail along with specimens of *Balanoglossus* were found devouring the latter alive. This was so unlike the former behavior that one was tempted to wonder whether they might have peculiarities of diet, and that their habitat on these sand flats, where likewise *Balanoglossus* has its home, might sustain some relation thereto. I therefore repeated the former experiment of offering them shreds of crab and fish meat and with the same negative results. I then tested them again with the *Balanoglossus* and found that it was taken quite readily by the same specimens which had refused the other bait. Leaving them for several days they were again tested with the same foods and with the same negative results. Having no specimens of *Balanoglossus* at hand some annelids, *Hydroides*, were offered them alive, and they were readily taken by three out of four tested.

No further qualitative tests were made along this line, but it would seem as if they were rather partial as to feeding habits, and particularly as to whether it be living or otherwise.

Limited tests were made as to their reaction to such substances as blood of crabs, clams, etc., but there seemed hardly any definite reactions indicative of olfactory, or gustatory sensibility. The swallowing reaction of *Eloactis* is much as in other species of actinians, namely that it consists largely of oral efforts. The tentacles play but little part in the reaction, though serving to press the food down upon the oral margins or lips. The swallowing act in these creatures involves something of a peristalsis of the esophagus. It was observed in several instances that any considerable irritation of a specimen during the swallowing process was almost invariably followed by a reversion and ejection of the food. A worm three fourths swallowed would be ejected by a sort of antiperistalsis, which was more rapid than the swallowing had been.

From what has just been stated it need hardly be observed that attempts to feed specimens with bits of blotting paper, or other such materials, were uniformly negative in character.

The feeding experiments with other species were too limited to justify any special attention in this connection. In most cases no difficulty was encountered in inducing species of *Sagartia* to take food of almost any sort.

Burrowing Reaction. — Attention has been directed in an earlier connection to the fact that considerable variability is evident among various specimens as to the matter of burrowing, or tube-building. It may not be without some interest to briefly cite a few details along this line. It is one of the curious features in the activities of *Eloactis* that among a dozen specimens put into an aquarium the most remarkable difference of behavior in this respect may be seen. Most will show early signs of activity, and soon bury their bodies as completely as possible, and assume an erect position. Others appear to go through the efforts but in a most futile way. Left over night the aquarium will show in the tracks over the surface of the sand the varied movements made in this way. Still other specimens seem to show no effort whatever to burrow, but lie indifferently upon the surface, hardly showing signs of life except as they are stimulated by some means. This may continue somewhat indefinitely. But after a time a change may come over one of these sluggish specimens and it sets about constructing a burrow all at once, as it were, and within a night will have taken up the characteristic attitude of its kind. If now it be dug out and left again upon the sand it may promptly readjust itself again in a burrow, or it may remain for some days in the same indifferent aspect. Specimens which first bury themselves are usually prompt to build fresh burrows if dug out of the earlier ones.

The facts herein portrayed suggest several interesting inferences and inquiries by way of conclusion.

1. It seems clear that in the behavior of actinians toward light one is forced to recognize that certain species have sensory perceptions of photic stimuli quite as well defined as exist in such organisms as the earthworm, clam, etc. And while in this group of cœlenterates no such definite sensory organs are known as those found in many medusæ, the Hertwigs have described certain ectodermal cells which they have designated as sensory in function. It is not without some warrant that we may conclude that the various aspects of behavior under consideration are more or less definitely correlated with sensory structures and perhaps nerve cells.

2. Loeb, who has studied certain aspects of the behavior of *Cerianthus membranaceus* (" Physiology of the Brain," pp. 56–59), attributes them to the influence of two tropic forces, namely, geotropism and heliotropism. " Positive geotropism and positive stereotropism cause the Ceriantbi to burrow in the sand vertically, and positive geotropism keeps them permanently in the burrow."

I have elsewhere shown the inadequacy of this explanation as applied to tube-dwelling annelids. I believe the facts under review may likewise be better understood and more consistently explained by other modes. Certainly the factor of light must be reckoned with as potent in the behavior of the several species studied. Again the variable behavior of these creatures in their burrowing habits is not easily accounted for on the usual theory of tropisms. Furthermore, it seems highly probable that in some cases the food-taking habit may sustain a relation to the general tube-dwelling habit.

3. Finally, as one considers the interesting facts as to the distribution of these light-reacting anemones the foregoing inferences are strongly corroborated. It is not necessary to review these facts in detail. It will be recalled that the observations of Quatrefages and Haime, already cited, had to do with species of *Cerianthus* and *Edwardsia* both of burrowing habit. Those of the Hertwigs were made on a species of *Cladactis*, an inhabitant of the deep sea. The observations of Jourdan were made on a species of *Paractis*, whose habit is not given, though species of this genus taken by the Challenger Expedition were also from the deep sea.

Of the species which have come under my own observations as light-perceptive, two are tube-dwelling, and one free-living, but secreting itself under various forms of cover, or occasionally burrowing in sand. Certainly neither geotropism nor stereotropism are equally or reasonably applicable as explanations of all these varied conditions and habits. I believe we are therefore forced to the conclusion that physiological conditions of adaptation are primarily involved, and that the various phases of behavior are so many expressions of such adjustments.

Syracuse University,
January 1, 1907.

ON THE ANATOMY OF THE CENTRAL NERVOUS SYSTEM OF THE NINE-BANDED ARMADILLO (TATU NOVEMCINCTUM LINN.).[1]

LILY C. SHUDDEMAGEN.

The present paper is a contribution to the macroscopical anatomy of the brain, spinal cord, cranial and spinal nerves of the nine-banded armadillo.

There appears to be no literature on any portion of the central nervous system except the brain in any edentate, with the exception of Pouchet's classic account of *Myrmecophaga;* with this omission the relations of the spinal cord in this group are unknown, and the present description of that organ complex is an attempt to fill this hiatus in our knowledge. A list of the memoirs treating of the anatomy of the brain is appended at the end of the present paper; of those memoirs, the ones by Gervais (1869), Pouchet (1869), and notably Smith (1899) are the most important. Smith is the only writer who mentions the brain of the particular species examined by me, but gives no figures of it; and indeed, our knowledge of the general anatomy of this species is much more scant than of various other armadillos,— even the rare *Chlamydophorus.*

Comparisons of the brain of this species are made with the brains of other described Dasypodidæ, and I have followed Smith's nomenclature of the parts.

The material used consisted of four specimens, two males and two females, procured in the neighborhood of Austin, Travis County, Texas. Two of these were preserved in formalin and two in alcohol.

This work has been done entirely under the direction of Prof. Thos. H. Montgomery, Jr., and the writer is under great obligation to him for his helpful suggestions, and kindly sympathy and constant encouragement during the preparation of this memoir.

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 75.

1. The Brain.

General Topography.

The brain is almost twice as long as broad. The bulbus olfactorius forms the most anterior, and the medulla oblongata its most posterior parts. The cerebrum broadens out posteriorly, and, on lateral view, is not quite as high as the cerebellum. The general shape is much like that of the lower mammals.

(*a*) *Prosencephalon.* — The prosencephalon is composed of the following parts : bulbus olfactorius, tuberculum olfactorium, lobus pyriformis, pedunculus olfactorius, locus perforatus, and the cerebral hemispheres.

The *bulbus olfactorius* (Pl. XXIV., Fig. 1, *Bul. Olf.*) is the most anterior part of the brain, and is relatively enormous. From a ventral view, it is seen to be heartshaped with the apex pointing forward. The ventral surface is indented by almost parallel furrows or sulci, running at right angles to the long axis of the brain. The dorsal surface (Fig. 4) is spherically rounded and smooth. It is placed somewhat ventral to the cerebral hemispheres (Fig. 2), so that over half of its dorsal surface is overlapped by them. From the anterior part of the bulbus olfactorius the olfactory nerve spreads out in a great fan-shaped mass.

The *tuberculum olfactorium* (Pl. XXIV., Fig. 1, *Tub. Olf.*) is a large oval area slightly raised above the surrounding regions. It is separated from the bulbus olfactorius by the pedunculus olfactorius. Its surface is not smooth, but somewhat tuberculated. It reaches a relatively large size in the armadillo.

The *lobus pyriformis* is visible along the lateral surface of the brain, just posterior to the tuberculum olfactorium ; it consists of an anterior lobe (*Lob. Pyr. A.*, Fig. 2), and a posterior (*Lob. Pyr. P.*, Fig. 1).

The *pedunculus olfactorius* (Pl. XXIV., Fig. 3, *Ped. Olf.*) is to be seen only in a lateral view of the brain. It connects the bulbus olfactorius with the remainder of that organ. In both dorsal (Pl. XXIV., Fig. 4), and ventral (Fig. 1), views it is hidden by the cerebral hemispheres and the oblique position of the bulbus olfactorius.

The *locus perforatus* (Pl. XXIV., Fig. 1, *Loc. Perf.*) is the depressed, quadrilateral area immediately anterior to the optic chiasma.

The *cerebral hemispheres* (Pl. XXIV., Figs. 2 and 4) show as high a development as any of the armadillos figured by Smith. The short *anterior rhinal fissure* (Pl. XXIV., Fig. 4, *Fis. Rh. A.*) begins in the boundary between the bulbus olfactorius and the hemispheres. It extends obliquely upwards for about a fourth of the length of the hemispheres. The *posterior rhinal fissure* (Pl. XXIV., Fig. 4, *Fis. Rh. P.*) begins near the posterior border of the hemispheres and runs horizontally towards the anterior part of the hemispheres, where it joins the sulcus β (Pl. XXIV., Fig. 4, β). In the most dorsal part of the hemispheres, the sulci γ and δ (Pl. XXIV., Fig. 4) are faintly developed. The latter of these two sulci corresponds to the suprasylvian sulcus of other mammals. On the mesial surface of the two hemispheres the sulcus limitans pallii (Pl. XXIV., Fig. 3, *Sul. L.*) is found.

In this animal, as in all mammals, a series of nerve fibers, or *commissures*, serve to connect homologous areas of the two hemispheres.

The most dorsally placed commissure is in the form of an inverted, obliquely placed U (Pl. XXIV., Fig. 3, *Cor. Cal.*). The arms of the U are formed by the corpus callosum (Pl. XXIV., Fig. 3, *Cor. Cal.*), and the ventral and dorsal psalterium (Pl. XXIV., Fig. 3, *Psal. V.* and *D.*). The curve of the U is formed by the splenium (Pl. XXIV., Fig. 3, *Spl.*). This commissure is placed more nearly vertical, and is rather smaller than in most of the edentate brains figured by Smith. There is really no apparent distinction between the dorsal and ventral psalterium. The psalterium is slightly longer than the corpus callosum. The two arms of this dorsal commissure are in contact with each other for the greater part of their extent, only the most ventral part of the psalterium extends a little further ventrally than the corpus callosum. The interval between the two arms of the dorsal commissure is called the septum lucidum in human anatomy. In the edentates, Smith calls this the paracommissural body. But since the two arms of the commissure are in contact with each other for the greater part of their extent, there is practically no septum lucidum or paracommissural body, in this armadillo.

The *anterior commissure* (Pl. XXIV., Fig. 3, *Com. A.*) is of fairly large size. It is a rather cylindrical bundle of fibers and connects the pyriform lobes. Because of the relatively large size of the pyriform lobes, the anterior commissure attains its increase of size. In the armadillos, all the parts of the brain connected with the sense of smell, reach relatively large dimensions.

(*b*) *Thalamencephalon.* — The thalamencephalon is the second embryological division of the brain, and consists of that part which bears the optic thalami, the infundibulum, pituitary body, and pineal body.

The *optic thalami* (Pl. XXV., Fig. 7, *Opt. Th.*) and the corpora quadrigemina (Pl. XXV., Fig. 7, *Cor. Q.*) form a large area of quadrilateral shape. The optic thalami are separated from each other, in the median line, by the third ventricle. They are connected across this ventricle by means of the commissura molli. This extends across the slit-like third ventricle as a large cylindrical mass of fibers (Pl. XXIV., Fig. 3, *Com. Mol.*). Thus the third ventricle becomes reduced to a narrow circular channel surrounding the commissura molli.

The floor of the third ventricle is drawn downward into a funnel-shaped pouch, the *infundibulum* (Pl. XXIV., Fig. 3, *Inf.*).

The *hypophysis* (Pl. XXIV., Fig. 3, *Hyp.*) is attached to the ventral part of the infundibulum.

The *pineal body* (Pl. XXV., Fig. 7, *Cor. Pin.*) lies in a shallow groove of the anterior corpora quadrigemina, just posterior to the third ventricle.

The *third ventricle* (Pl. XXV., Fig. 7, *Ven. III*) opens into the two first ventricles (Pl. XXV., Fig. 7, *Ven. I*) by means of the foramen of Monroe (Pl. XXV., Fig. 7, *For. M.*). Out of the posterior part of the third ventricle, the aqueduct of Sylvius (Pl. XXIV., Fig. 3, *Aq. Syl.*) opens and passes into the fourth ventricle.

The *II, or optic nerve* (Pl. XXIV., Fig. 1, *II*) comes off from the ventral surface of the brain, just a little anterior to the infundibulum. It is of very small size, because of the great diminution of the visual acuteness and consequent reduction of the size of the eye.

The *IV, or pathetic nerve* (Pl. XXIV., Fig. 1, *IV*) arises from the ventral surface of the brain, just posterior to the infundibulum.

(*c*) *Mesencephalon.* — The mesencephalon is that embryological division of the brain which gives rise to the corpora quadrigemina and the crura cerebri.

The *corpora quadrigemina* (Pl. XXV., Fig. 7, *Cor. Q.*) lie immediately posterior to the optic thalami. The anterior pair of the corpora quadrigemina forms an area slightly elevated above the level of the optic thalami. Just posterior to them, the posterior pair of the corpora quadrigemina rise to a much higher level (Pl. XXIV., Fig. 3, *Cor. Q.*) ; their most dorsal point comes up almost to the level of the cerebral hemispheres. The corpora quadrigemina are wedged between the cerebellum and the cerebral hemispheres. In the armadillo, they are not separated across the middle, but form one body in which separation is only faintly indicated by a shallow longitudinal furrow. The reduction in the size of the anterior pair of the corpora quadrigemina is probably due to the waning importance of the sense of sight. The posterior corpora quadrigemina retain their large size, or perhaps even show an increase in size, because they are not connected as directly with the sense of sight.

The *crus cerebri* arises from under the optic tract as a faint, indistinct band of fibers, runs backwards and disappears under the pons Varolii.

(*d*) *Metencephalon.* — The embryological division of metencephalon gives rise, in the adult, to the cerebellum.

Viewed dorsally (Pl. XXIV., Fig. 4), the cerebellum presents a somewhat triangular shape, where the paraflocculi (Pl. XXIV., Fig. 4, *Par. Fl.*) and the posterior lobe (Pl. XXIV., Fig. 4, *Lob. P.*) form the three angles. The cerebellum is much convoluted, as is the case in all mammals. Its greatest diameter is transverse. This large cerebellar mass hides from view the entire fourth ventricle except the most posterior part (Pl. XXIV., Fig. 4, *Ven. IV*). The cerebellum is supported and connected with the brain stem by two cerebellar peduncles (Pl. XXV., Fig. 7, *Ped. Cer.*). Anteriorly, the cerebellum is closely adapted to the contour of the cerebral hemispheres. It projects forward sufficiently to hide the posterior corpora quadrigemina completely.

The most lateral projections of the cerebellum are two fairly large sized bodies. These bodies, composed of a number of folia and separated almost entirely from the remainder of the cerebellum by a fissure, are the *lobi flocculi.*

Each of these *lobi flocculi* consists of two distinct parts, the flocculus (Pl. XXIV., Figs. 1 and 2, *Floc.*) and the paraflocculus (Pl. XXIV., Figs. 2 and 4, *Par. Fl.*). The latter is much the largest of the two, and almost completely hides the former from view. From a dorsal view the paraflocculus (Pl. XXIV., Fig. 4, *Par. Fl.*) appears as a crescentic mass of folia, forming the lateral projections of the cerebellum.

Aside from the *lobi flocculi,* the remainder of the cerebellum may be divided into three lobes, the lobus anticus (Pl. XXIV., Fig. 4, *Lob. A.*), the lobus centralis (Pl. XXIV., Fig. 4, *Lob. C.*), and the lobus posticus (Pl. XXIV., Fig. 4, *Lob. P.*).

The *lobus anticus* (Pl. XXIV., Figs. 2, 3, and 4, *Lob. A.*) is separated from the posterior part of the cerebellum by the fissura prima (Pl. XXIV., Figs. 2, 3, and 4, *Fis. 1*). It is clearly visible in a dorsal view of the brain (Pl. XXIV., Fig. 4, *Lob. A.*), and is not hidden between the lobus centralis and the cerebral hemispheres, as is the case in the *Chlamydophorus* (Smith, 1899, Fig. 34) or in *Xenurus* (Smith).

The *lobus centralis* (Pl. XXIV., Figs. 2, 3 and 4, *Lob. C.*) is separated from the lobus posticus by means of the fissura secunda (Pl. XXIV., Figs. 2, 3 and 4, *Fis. 2*). It constitutes the largest and most complex part of the cerebellum. It is a large irregular area which has bulged forward and laterally, wedging its way between the lobus anticus and the lobus flocculus.

The *lobus posticus* (Pl. XXIV., Figs. 2, 3 and 4, *Lob. P.*) is the most caudal part of the cerebellum. It is small, consisting of but few folia, and covers over almost completely the posterior part of the fourth ventricle.

(*e*) *Myelencephalon.* — The embryonic division of myelencephalon gives rise, in tne adult, to the medulla oblongata and the pons Varolii.

The *medulla oblongata* (Pl. XXIV., Fig. 4, *Med. Obl.*) is the most posterior part of the brain, and is continued directly into the spinal cord. In the medulla oblongata is the fourth ventricle, roofed over by a thin membrane. The greatest part of the medulla oblongata is covered over by the cerebellum.

The *pons Varolii* (Pl. XXIV., Fig. 3, *Pons*) forms the most anterior part of the hind brain. It is a pair of slight elevations on the ventral surface of the brain, a little posterior to the infundibulum.

From the medulla oblongata arise all the remainder of the cranial nerves, from the V to the XII inclusive.

The *V, or trigeminal nerve* (Pl. XXIV., Fig. 1, *V*) arises from the pons Varolii. It soon divides into two branches, the most lateral of which subdivides again.

The *VI, or abducent nerve* (Pl. XXIV., Fig. 1, *VI*) arises in the region of the pons Varolii, and runs to the external rectus eye muscle.

The *VII, or facial nerve* (Pl. XXIV., Fig. 1, *VII*) arises in close connection with the VIII nerve, in the region just laterad of the pons Varolii. It soon subdivides into branches.

The *VIII, or auditory nerve* (Pl. XXIV., Fig. 1, *VIII*) arises with the VII nerve from the same part of the brain. It runs directly outwards and enters the cochlea of the ear.

The *IX, or glosso-pharyngeal nerve* (Pl. XXIV., Fig. 1, *IX*) arises by several roots, from the ventral surface of the medulla.

The *X, or pneumogastric nerve* (Pl. XXIV., Fig. 1, *X*) arises by several roots from the medulla, just posterior to the IX nerve.

The *XI, or spinal accessory nerve* (Pl. XXIV., Fig. 1, *XI*) arises by several roots from the ventral surface of the medulla and the spinal cord. Some of its roots arise from the spinal cord, as far back as the fourth cervical nerve.

The *XII, or hypoglossal nerve* (Pl. XXIV., Fig. 1, *XII*) arises by several roots from the medulla oblongata, just posterior to the origin of the XI nerve.

2. Spinal Cord.

The spinal cord is cylindrical, but somewhat flattened dorsoventrally. In the cervical and sacral regions, it has a slight enlargement from which the nerves of the brachial and lumbosacral plexuses are given off. In the sacral region the cord breaks up into a number of fine nerves which occupy the vertebral canal as the cauda equina. These nerve branches pass out, pair by pair, from between the caudal vertebræ and supply the muscles of the tail.

The most anterior division of the spinal nerves is the cervical (Pl. XXVI., *C. 1–C. 8*). Of these there are eight pairs.

Of the thoracic nerves (Pl. XXVI., *T. 1–T. 10*) there are ten pairs.

The lumbar region is very short, containing six pairs of nerves (Pl. XXVI., *L. 1–L. 6*).

The sacral nerves (Pl. XXVI., *S. 1–S. 8*) are eight in number.

The exact number of the caudal nerves was not ascertained by me. But they are quite numerous, possibly as many as fifteen to twenty pairs.

(*a*) *Cervical Plexus.* — The cervical plexus (Pl. XXVI., *C. 1–C. 8*) is composed of the dorsal branches of the eight pairs of cervical nerves. These branches pass almost vertically upwards, interlace, and supply the dorsal neck muscles. On Pl. XXVI., on the right hand side of the drawing, are shown the dorsal branches of the cervical nerves.

(*b*) *Brachial Plexus.* — The brachial plexus is composed of the large ventral branches of the third, fourth, fifth, sixth, seventh, and eighth cervical nerves, and the first and second thoracic nerves. The formation of the plexus is due to the union of the several nerves, by means of strong connecting branches. The plexus lies in the axilla, and all the component nerves pass out laterally, almost parallel to the first rib. By means of its branches, the arm and shoulder are innervated.

The three *subscapular nerves*, the cranial (Pl. XXVI., *Sub. Sc. 1*), the middle (Pl. XXVI., *Sub. Sc. 2*), and the caudal (Pl. XXVI., *Sub. Sc. 3*), all supply muscles on the ventral surface of the scapula. The cranial subscapular nerve (Pl. XXVI., *Sub. Sc. 1*) arises from the third, fourth, and fifth cervical nerves. The middle subscapular nerve (Pl. XXVI., *Sub. Sc. 2*) arises from the sixth cervical nerve. The caudal subscapular nerve (Pl. XXVI., *Sub. Sc. 3*) arises from the sixth, seventh, and eighth cervical nerves.

The *suprascapular nerve* (Pl. XXVI., *Sup. Sc.*) arises from the fifth cervical nerve. It passes onto the dorsal side of the scapula and enervates the supraspinatus and infraspinatus muscles.

The *axillary nerve* (Pl. XXVI., *Ax.*) arises from the fifth and sixth cervical nerves. It supplies some muscles in the upper arm.

The *radiales nerve* (Pl. XXVI., *Rad.*) is one of the three nerves that supply the lower arm and hand. It arises from the sixth, seventh, and eighth cervical nerves.

The *medianus nerve* (Pl. XXVI., *Med.*) also principally supplies the muscles of the forearm and hand. It arises from the seventh, and eighth cervical, and the first thoracic nerves.

The *ulnaris nerve* (Pl. XXVI., *Uln.*) is the third lower arm and hand nerve. It arises from the eighth cervical, and first and second thoracic nerves.

(*c*) *Thoracic Plexus.*— From the first, second, and third thoracic nerves arise three ventral branches which pass out laterally and unite into a little separate plexus (Pl. XXVI., *X.*) Then this plexus gives off three main branches which subdivide again and again. All of these branches supply the great lateral skin muscle which is attached along the whole length of the armor. A plexus like this, to my knowledge, is not present in any other mammal. It has probably arisen because of the great development of the large skin muscle, which attaches to the sides of the armor and functions in drawing the animal together in a ball. Because of its origin from the thoracic nerves, I have taken the liberty of naming it the thoracic plexus.

The remainder of the thoracic nerves are arranged similarly to those of other mammals. They divide into two branches almost immediately after leaving the intervertebral foramina. The dorsal branches supply the superficial muscles of the back, while the ventral branches run along the ribs as the intercostal nerves.

(*d*) *Lumbar Nerves.* — The first three lumbar nerves take no part in the formation of the lumbo-sacral plexus. The ventral branch of the first lumbar nerve divides into two branches, the ilio-hypogastric (Pl. XXVI., *Il. Hyp.*), and the ilio-inguinal (Pl. XXVI., *Il. Ing.*). The ventral branch of the second lumbar nerve forms the genito-crural nerve (Pl. XXVI., *Gen. Cr.*). The third lumbar nerve forms the external cutaneous nerve (Pl. XXVI., *Ext. Cut.*).

(*e*) *Lumbo-Sacral Plexus.* — The lumbo-sacral plexus is composed of the fourth, fifth, and sixth lumbar, and the eight sacral nerves. These nerves are all interconnected by strong branches, and they supply the muscles of the thigh and lower limb.

The *anterior crural nerve* (Pl. XXVI., *Ant. Cr.*) is composed of parts of the fourth, fifth, and sixth lumbar nerves. It supplies some of the upper thigh muscles.

The *obturator nerve* (Pl. XXVI., *Obt.*) arises from the sixth lumbar and first sacral nerves. It also goes to supply some of the upper thigh muscles.

The *sciatic major nerve* (Pl. XXVI., *Sc. Maj.*) arises from the sixth lumbar, and first, second, and third sacral nerves. This is the great nerve of the posterior limb. It soon divides into the tibialis (Pl. XXVI., *Tib.*), the perroneus (Pl. XXVI., *Per.*), the gluteous (Pl. XXVI., *Glut.*), and the sciatic minor nerve (Pl. XXVI., *Sc. Min.*).

The *pudendus nerve* (Pl. XXVI., *Pud.*) arises from the fourth sacral nerve.

The *cutaneous femoris nerve* (Pl. XXVI., *Cut.*) arises from the fifth sacral nerve.

General Remarks.

The brain has been previously described for the following Dasypodids :

The brain of *Chlamydophorus truncatus* has been figured and described by Smith (1899) and Pouchet (1869). Hyrtl (1855) gives just a few brief notes on the brain, without any figures.

Dasypus sexcinctus has been figured and described by Smith (1899), Turner (1867), and Pouchet (1869).

Priodon gigas has been figured by Pouchet (1868 and 1869), and mentioned by Smith (1899).

Tolypentes tricinctus has been mentioned by Smith (1899), and figured and described by Gervais (1869).

Tatu novemcinctum has been mentioned by Smith (1899), without figures.

Tatu peba has been figured and described by Smith (1899) and Rapp (1852).

Xenurus unicinctus has been figured and mentioned by Smith (1899) and Garrod (1878).

Dasypus villosus has been figured and described by Smith (1899).

Smith's (1899) work on the Armadillos is by far the most important, and for this reason I have compared the species under present consideration with his descriptions.

The brain of *Tatu novemcinctum* shows less similarity with the genus *Chlamydophorus*, than with the brain of any other genus of armadillo. To judge by the figure of *Xenurus unicinctus* given by Garrod (1878), there seems to be greater similarity of the brain of *Tatu* with *Xenurus* than with any other genus of armadillo. The fissures, sulci, and the general shape and contour of these two brains have very many points in common. However, much more detailed study must be made of all the species of *Armadillo* before one could venture to assert this with any degree of certainty.

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DESCRIPTION OF THE PLATES.

The following abbreviations have been used:

(Greek letters denote sulci.)

Ant. Cr.	anterior crural.
Aq. Syl.	aqueductus Sylvii.
Ax.	axillary.
Bul. Olf.	bulbus olfactorius.
C. 1–8.	cervical nerves.
Com. A.	commissura anterior.
Com. Mol.	commissura mollis.
Com. P.	commissura posterior.
Cor. Cal.	corpus callosum.
Cor. Pin.	corpus pineale.
Cor. Q.	corpora quadrigemina.
Cut. Fem.	cutaneus femoris posterior.
Ex. Cut.	external cutaneous.
Fis. 1.	fissura prima.
Fis. 2.	fissura secunda.

Fis. Rh. A.	anterior rhinal fissure.
Fis. Rh. P.	posterior rhinal fissure.
Floc.	flocculus.
For. M.	foramen of Monro.
Gen. Cr.	genito-crural.
Glut.	glutæus inferior.
Hipp.	hippocampus.
Hyp.	hypophysis.
Il. Hyp.	ilio-hypogastric.
Il. Ing.	ilio-inguinal.
Inf.	infundibulum.
L. 1–10.	lumbar nerves.
Lob. A.	lobus anticus.
Lob. C.	lobus centralis.
Lob. P.	lobus posticus.
Lob. Pyr. A.	lobus pyriformis anterior.
Lob. Pyr. P.	lobus pyriformis posterior.
Loc. Perf.	locus perforatus.
Med.	medianus.
Med. Obl.	medulla oblongata.
Obt.	obturator.
Opt. Th.	optic thalami.
Par. Fl.	paraflocculus.
Ped. Cer.	pedunculi cerebelli.
Ped. Olf.	pedunculus olfactorius.
Per.	peroneus.
Psal. D.	psalterium dorsale.
Psal. V.	psalterium ventrale.
Pud.	pudendus.
Rad.	radialis.
S. 1–8.	sacral nerves.
Sc. Maj.	major sciatic.
Sc. Min.	minor sciatic.
Spl.	splenium.
Sub. Sc. 1.	cranial subscapularis.
Sub. Sc. 2.	middle subscapularis.
Sub. Sc. 3.	caudal subscapularis.
Sul. L.	sulcus limitans pallii.
Sup. Sc.	suprascapularis.
T. 1–10.	thoracic nerves.
Tib.	tibialis.
Tr. Opt.	tractus opticus.
Tub. Ac. L.	tuberculum acusticum laterale.
Tub. Ac. M.	tuberculum acusticum median.
Tub. Olf.	tuberculum olfactorium.
Uln.	ulnaris.
Ven. I.–IV.	ventricles I.–IV.
I.–XII.	cranial nerves.

Explanation of Plates.

All the figures are from enlarged freehand sketches. The figures on Plates I. and II. were drawn twice natural size, and then reduced about one third in the reproduction. Plate III. was drawn natural size, and then reduced about one half in the reproduction.

Plate XXIV.

Fig. 1. Ventral view of the brain.
Fig. 2. Lateral view.
Fig. 3. Median longitudinal section.
Fig. 4. Dorsal view.
Fig. 5. Ventral view of a late fœtal brain ; length of the fœtus was about 15 cm.
Fig. 6. Dorsal view of the same late fœtal brain as Fig. 5.

Fig. 1

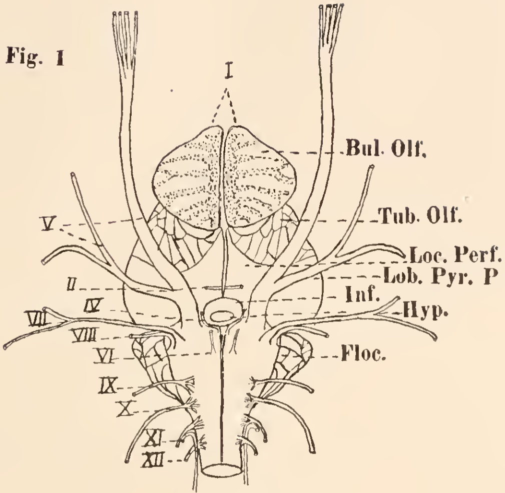

I
Bul. Olf.
Tub. Olf.
V
Loc. Perf.
Lob. Pyr. P
II
Inf.
IV
Hyp.
VII
VIII
Floc.
VI
IX
X
XI
XII

Fig. 6

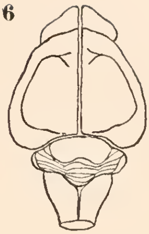


Fig. 5

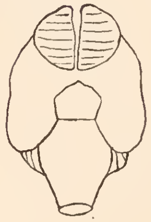


Fig. 2

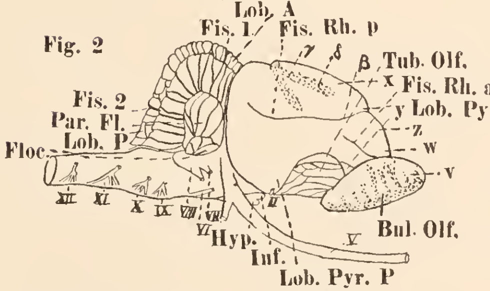

Lob. A
Fis. 1
Fis. Rh. p
γ
δ
β
Tub. Olf.
x
Fis. Rh. a
y
Lob. Pyr. A
z
w
v
Fis. 2
Par. Fl.
Lob. P
Floc.
XII
XI
X
IX
VIII
VII
VI
II
Hyp.
Inf.
V
Bul. Olf.
Lob. Pyr. P

Fig. 3

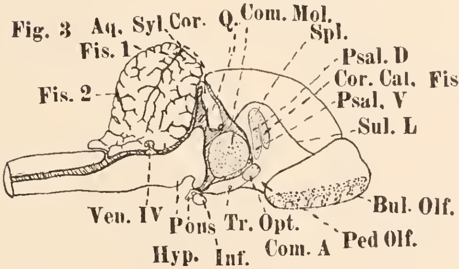

Aq. Syl.
Cor. Q.
Com. Mol.
Spl.
Fis. 1
Psal. D
Cor. Cal.
Fis. 2
Psal. V
Sul. L
Bul. Olf.
Ven. IV
Pons
Tr. Opt.
Ped Olf.
Hyp.
Inf.
Com. A

Fig. 4

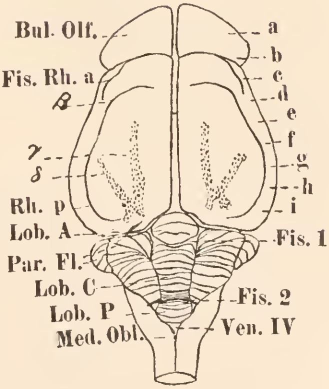

Bul. Olf.
a
b
Fis. Rh. a
c
β
d
e
f
γ
g
δ
h
Fis. Rh. p
i
Lob. A
Fis. 1
Par. Fl.
Lob. C
Fis. 2
Lob. P
Ven. IV
Med. Obl.

PLATE XXV.

FIGS. 7–11. Horizontal, longitudinal sections of the brain. The dotted parts of the figures show the ventricles.

FIG. 7. The dorsal surface of a horizontal section; taken in plane *v* of Fig. 2.

FIG. 8. The dorsal surface of a horizontal section, taken in the plane *w* of Fig. 2.

FIG. 9. The dorsal surface of a horizontal section, taken in the plane *z* of Fig. 2.

FIG. 10. The dorsal surface of a horizontal section, taken in the plane *y* of Fig. 2.

FIG. 11. The dorsal surface of a horizontal section, taken in plane *x* of Fig. 2.

FIGS. 12–20 are cross-sections of the brain, beginning at the anterior end.

FIG. 12. The caudal surface of a cross-section taken in the plane *a* of Fig. 4.

FIG. 13. The caudal surface of a cross-section taken in the plane *b* of Fig. 4.

FIG. 14. The caudal surface of a cross-section taken in the plane *c* of Fig. 4.

FIG. 15. The caudal surface of a cross-section taken in the plane *d* of Fig. 4.

FIG. 16. The caudal surface of a cross-section taken in the plane *e* of Fig. 4.

FIG. 17. The caudal surface of a cross-section taken in the plane *f* of Fig. 4.

FIG. 18. The caudal surface of a cross-section taken in the plane *g* of Fig. 4.

FIG. 19. The caudal surface of a cross-section taken in the plane *h* of Fig. 4.

FIG. 20. The caudal surface of a cross section taken in the plane *i* of Fig. 4.

Fig. 7

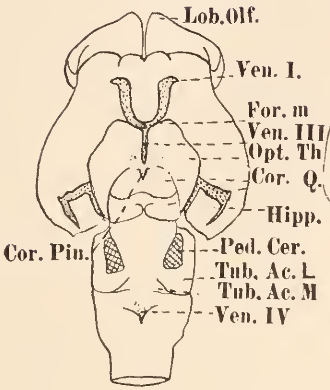

v
Lob. Olf.
Ven. I.
For. m
Ven. III
Opt. Th
Cor. Q.
Hipp.
Cor. Pin.
Ped. Cer.
Tub. Ac. L
Tub. Ac. M
Ven. IV

Fig. 8

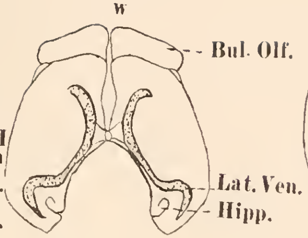

w
Bul. Olf.
Lat. Ven.
Hipp.

Fig. 9

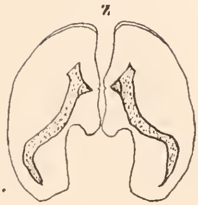


Fig. 10

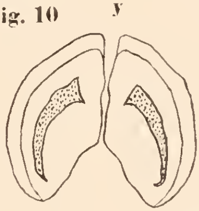


Fig. 11

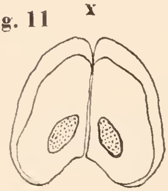


Fig. 12

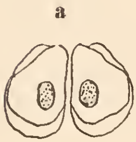


Fig. 13

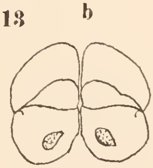


Fig. 14

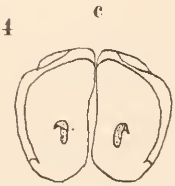


Fig. 15

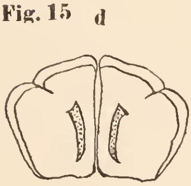


Fig. 16

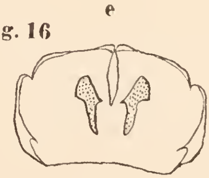


Fig. 17

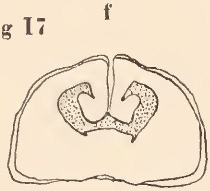


Fig. 18

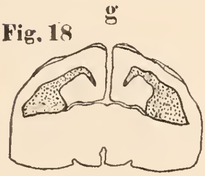


Fig. 19

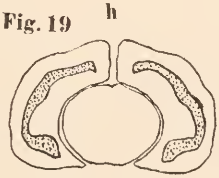


Fig. 20

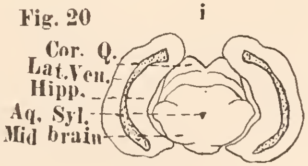
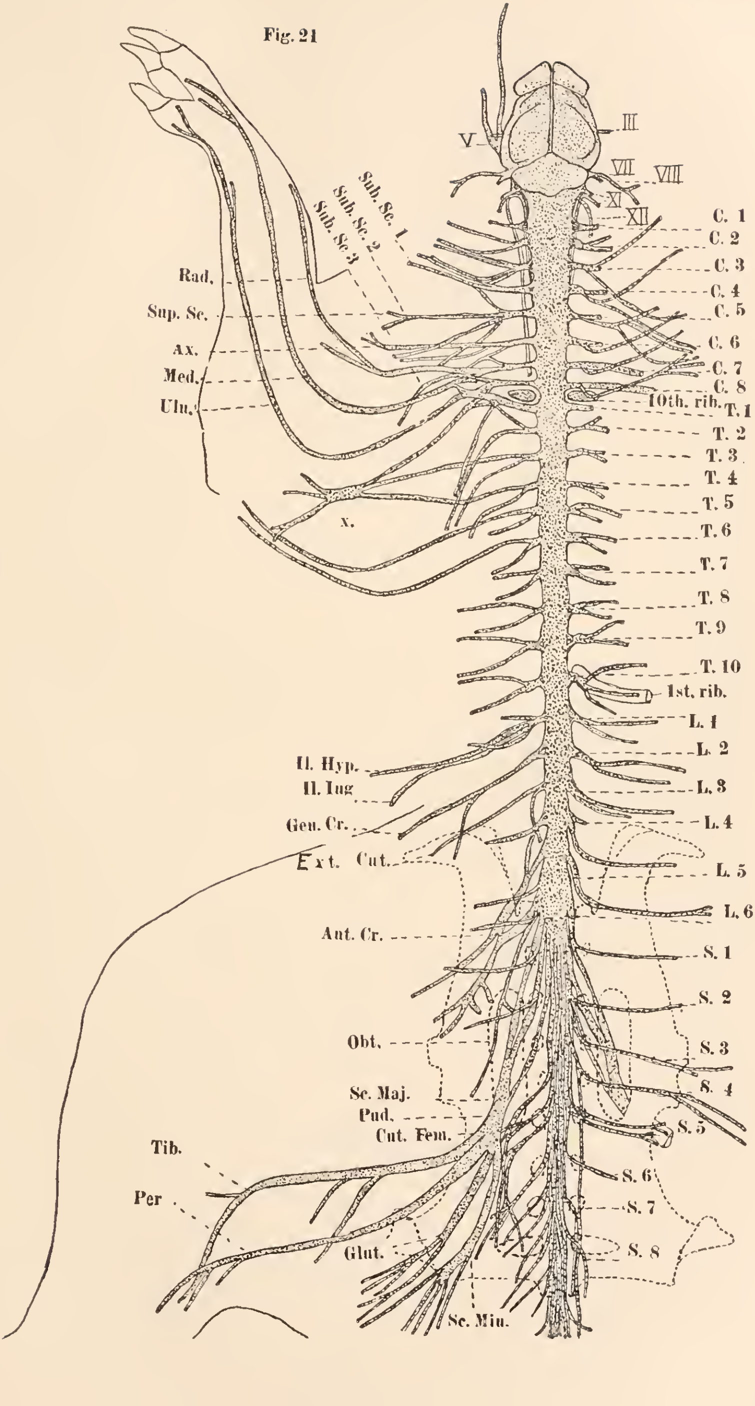

i
Cor. Q.
Lat. Ven.
Hipp.
Aq. Syl.
Mid brain

PLATE XXVI.

FIG. 21. A drawing of the entire central nervous system. The dotted lines show the outline of the sacrum in its natural relation to the spinal nerves. The nerves on the left hand side of the figure are all the ventral branches of the spinal nerves. On the right hand side of the figure, the more superficial branches of the spinal nerves are shown.

Fig. 21


V
III
VII
VIII
XI
XII
Sub. Sc. 1
Sub. Sc. 2
Sub. Sc. 3
C. 1
C. 2
C. 3
C. 4
C. 5
C. 6
C. 7
C. 8
Rad.
Sup. Sc.
Ax.
Med.
Uln.
10th. rib.
T. 1
T. 2
T. 3
T. 4
T. 5
T. 6
T. 7
T. 8
T. 9
T. 10
x.
1st. rib.
L. 1
L. 2
L. 3
L. 4
L. 5
L. 6
Il. Hyp.
Il. Ing.
Gen. Cr.
Ext. Cut.
Ant. Cr.
S. 1
S. 2
S. 3
S. 4
S. 5
S. 6
S. 7
S. 8
Obt.
Sc. Maj.
Pud.
Cut. Fem.
Tib.
Per
Glut.
Sc. Min.

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BIOLOGICAL BULLETIN

NOTE ON THE CHROMOSOME-GROUPS OF METAPODIUS AND BANASA.[1]

EDMUND B. WILSON.

I am led to publish the following preliminary note lest confusion should arise from the peculiar relations of the chromosomes in *Banasa calva* described in the second of my "Studies on Chromosomes,"[2] which have now, I believe, become explicable as a result of additional studies on *Banasa*, but more especially on the genus *Metapodius*. At the time these relations were described in *Banasa calva* they appeared to be unique in that both an unpaired chromosome (apparently an "accessory" chromosome) and a typical pair of unequal idiochromosomes are present in the same species, and four classes of spermatozoa are accordingly formed. The coexistence of these two forms of chromosomes in the same individual has already been considered by more than one writer as a serious difficulty in the way of my general interpretation of the significance of these chromosomes in sex-production. It was (and is) my view that the "accessory" chromosome is the homologue of the large idiochromosome and like the latter is distinctive of the female-producing spermatozoa. It seemed, no doubt, an obstacle to this view that an unpaired or heterotropic chromosome should coexist with a pair of idiochromosomes in the same species, and that it should in one class of spermatozoa be associated with a large idiochromosome, in another class with a small one. With the material at my command (which included only two testes from the Paulmier collection) I was not in a position to meet this difficulty or to give an adequate explanation of the facts; and for a time, I even suspected that the material might be pathological. Recently, however, I have found a similar, though not quite identical, condition in a species of *Metapodius*, and have been able to study the facts more thoroughly. In this form, too, an unpaired chromosome coexists with a typical pair of idiochromosomes (and a pair of *m*-chromosomes as well); but the facts clearly show that it is not of the same nature as the "accessory" or "heterotropic" chromosome of the usual type, and is without constant relation to sex-production. The idiochromosomes show the usual relation, the large one passing to the female producing pole and the small one to the male-producing pole. A comparison of different individuals shows beyond doubt that the unpaired chromosome may be either present or absent in either the male or female, and hence is without significance in sex-production. It is in fact a kind of supernumerary chromosome, which I shall designate as the "*s*-chromosome" in order to distinguish it from the odd sex-chromosome of the usual type — variously known as the "accessory chromosome" (McClung), "heterotropic chromosome" (Wilson), or "monosome" (Montgomery).

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[1] The material on which the observations were made is part of a series procured in the course of an extended collecting trip to the south and west in the summer of 1906, the cost of which was in part defrayed by a grant from the Carnegie Institution of Washington. The results will be published in a more extended form hereafter.

[2] *Journ. Exp. Zool.* II., 4, 1905.

1. METAPODIUS TERMINALIS Dall.[1]

The present account will give only the facts that bear directly on the case of *Banasa calva*. The genus *Metapodius* is, I believe, in a somewhat plastic condition as regards the chromosomes, and presents certain variations in the number of the larger chromosomes that need not here be described, since they do not affect the relations to be considered. Alone among all the Coreidæ thus far examined, the three species of *Metapodius* possess a typical pair of idiochromosomes along with a typical pair of *m*-chromosomes — a fact which proves the validity of the distinction between these two forms of chromosomes drawn in my second study. The idiochromosomes are distinctly, though not greatly, unequal in size ; and as usual among the Hemiptera, they remain separate as univalents in the first maturation division, but conjugate at the end of this division to form an unequal bivalent. In the greater number of individuals (which may be classed together as "Type A") the first division shows 13 chromosomes (Fig. 1, *b*) and the second 12. In the most usual arrangement the two idiochromosomes (*I* and *i*) lie in the first division not far apart, outside an irregular ring formed of nine larger bivalents, in the position typical of the odd chromosome in other coreids. Near the center of the ring lies a very small *m*-chromosome bivalent (*m*), which as in so many other cases is formed in the late prophases by conjugation of its two members. The thirteenth chromosome is the small unpaired univalent *s*-chromosome (*s*) which divides like all the others in the first division but passes undivided to one pole in the second division. In three of the seven males I have, this chromosome is of the same size as the *m*-chromosomes. In two individuals of the same type it is somewhat larger, though markedly smaller than the large bivalents. In the remaining two males (which constitute "Type B") the *s*-chromosome is wanting in all the cells, whether spermatogonia or spermatocytes. In these individuals the first spermatocyte division uniformly shows 12 chromosomes (Fig. 1, *c*) and the second 11, the grouping being otherwise more or less nearly similar to that in the first type.

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[1] The following description will be found to differ widely from that given for the same species by Montgomery (*Trans. Am. Phil. Soc.*, N. S., XXI., 3, 1906), who states that there are 21 spermatogonial chromosomes and an ordinary large odd chromosome in the second division. Professor Montgomery has kindly sent me some of his own material, collected in Pennsylvania, a study of which has convinced me of the correctness of his account. My own material is from New Jersey, North Carolina, South Carolina, Georgia and Ohio; and there can be no doubt of the identification since every original specimen is in my possession (as is the case with all my new material). Through the courtesy of Dr. Uhler I have been enabled to compare these specimens with those in his collection (with which they exactly agree); and they have also been examined by several competent hemipterists, including Mr. Otto Heidemann, of Washington, and Mr. H. G. Barber, of New York, and pronounced by them to be typical *terminalis*. As will be shown, different individuals among these specimens show constant and characteristic differences in the chromosome-groups; but none show less than 22 chromosomes, and none possess a large odd chromosome. The same is true of *M. femoratus* Fab., and *M. granulosus* Dall., both of which, like *terminalis*, possess a typical pair of idiochromosomes. This contradic-

tion probably cannot now be resolved, since the original specimens of Montgomery's material are not in existence. I think it probable that two different species have been under observation, and there is some reason to suspect that Montgomery's material may have been *Euthoctha galeator*. This case illustrates the extreme importance, in work of this kind, of preserving every individual from which cytological material is taken.

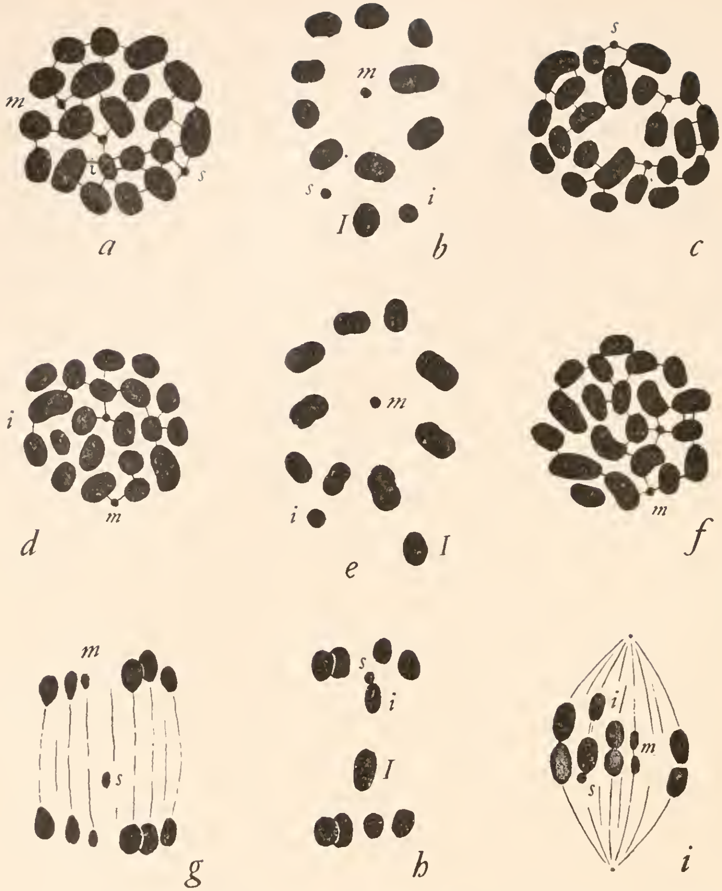

FIG. 1. *Metapodius terminalis.*[1] *a*, polar view, spermatogonial group, Type A; *b*, first maturation-division, Type A; *c*, female, Type A (probably a young follicle cell); *d*, spermatogonial group, Type B; *e*, first spermatocyte-division, Type B; *f*, female group, Type B; *g*, second division, Type A, showing *s*-chromosome free (the idiochromosomes do not appear in the plane of section); *h*, second division in side-view, *s*-chromosome coupled with small idiochromosome; *i*, second division, *s*-chromosome coupled with the large idiochromosome. (Fig. *a* is from a specimen taken at Madison, N. J.; *b*, *g*, *h*, *i*, from one individual from Charleston, S. C.; *c*, *e*, and *f* from Raleigh, N. C.; *d*, from Mansfield, Ohio. A second male of Type B was taken at Raleigh, N, C., from the same catalpa tree with an individual of Type A.)

[1] The enlargement is 3700 diameters — somewhat less than that of the figures in my preceding papers. The figures are all from camera drawings and are not schematized, except that in Fig. 1, *g*, one pair of the chromosomes has been slightly displaced in order to show the *m*-chromosomes more clearly. No attempt is made to show details of the achromatic spindles. In all the figures *I* denotes the large idiochromosome, *i*, the small one, *m* the *m*-chromosome, and *s* the *s*-chromosome. (The latter not to be distinguished from the *m*-chromosomes in *a* and *c*.)

When the *s*-chromosome is present it sometimes (in about 20 per cent. of the cases tabulated) lies free — *i. e.*, not connected with any other in either division — and after dividing in the first division, passes undivided to one pole in the second (Fig. 1, *g*). *In most cases it is in the second division attached to one or the other of the idiochromosomes and passes with it, undivided, to one pole* (Fig. 1, *h*, 1, *i*). In either case one pole receives 11 chromosomes and one 12, as may clearly be seen in polar views of the late anaphases which show both daughter-groups of chromosomes in the same spindle. Four classes of spermatozoa are accordingly formed in this type, which correspond to those described in *Banasa calva*. Designating the ordinary chromosomes or "allosomes" as "O," the large and small idiochromosomes respectively as "I" and "i," the m-chromosome as "m" and the s-chromosome as "s" the classes are as follows:

(1) $9\,O + I + m + s = 12,$
(2) $9\,O + I + m = 11,$
(3) $9\,O + i + m + s = 12,$
(4) $9\,O + i + m = 11.$

So far this is identical with the conditions described in *Banasa calva* except that in the latter case the unpaired chromosome fails to divide in the first division but divides in the second, while the reverse condition obtains in *Metapodius*. But there is now an important difference to consider which involves the most interesting phenomenon that occurs in this form. In *Banasa calva* the four classes are equal in number. In *Metapodius*, at least in certain individuals, this is not the case; for the *s*-chromosome shows a marked tendency to couple with the small idiochromosome rather than the large, which produces an excess of spermatozoa in which these two chromosomes are associated. It is somewhat difficult to secure adequate data, since the nature of the coupling can, as a rule, only be determined with certainty in side views of the middle anaphases. Out of 34 clear cases (taken from two individuals) the *s*-chromosome is coupled with the small idiochromosome in 24 and with the large in 10 — *i. e.*, in about 70 and 30 per cent. respectively, a ratio which may very likely be somewhat altered with a larger series of data. Of the four classes, accordingly, 2 and 3 are more numerous than 1 or 4.

Turning now to the spermatogonia, we find an accurate correlation between the spermatogonial chromosome-groups and those of the maturation divisions. In all cases there are 18 equally paired larger chromosomes, an unequal pair of idiochromosomes and a very small pair of *m*-chromosomes; and these 22 alone are present in the individuals of Type B (Fig. 1, *d*). In those of Type A an *s*-chromosome is present in addition, making 23 in all (Fig. 1, *a*). In three of the five males of this type, as stated above, the *s*-chromosome is no larger than the *m*-chromosomes, and the spermatogonia correspondingly show 20 large and three very small chromosomes. In the remaining two individuals of this type the *s*-chromosome is considerably larger than the *m*-chromosomes, both in the maturation-divisions and in the spermatogonia. The spermatogonia of these individuals seem therefore, at first sight, to show 21 large chromosomes and two small. In Type B, which have but 22 chromosomes, the first maturation-division shows but 12 chromosomes, the second 11; and only two classes of spermatozoa are formed, which correspond to Classes 2 and 4 of Type A.

The foregoing data, when compared with the conditions found in the female, give a decisive result regarding the relation of these chromosomes to sex-production. If the unpaired *s*-chromosome were of the same nature as the odd or "accessory" chromosome of other coreids we should expect to find one such chromosome in the male and two in the female; and since males and females alike possess in addition two small *m*-chromosomes the males should show three small chromosomes and the females four. Such however is not the case. In both sexes there are individuals that possess three small chromosomes (Fig. 1, *a*, 1, *c*) and others that possess but two (Fig. 1, *d*, 1, *f*). Evidently therefore the *s*-chromosome is indifferent as regards the sex-characters. On the other hand, close study of the larger chromosomes shows the same relations as those observed in other forms that possess unequal idiochromosomes. In the female groups all are equally paired. In the male all are thus paired save two, one of which is evidently the small idiochromosome.[1]

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[1] This fact is not always readily made out, since the small idiochromosome is not very markedly smaller than the others; but I am sure of the observation, and the fact was determined in many spermatogonial groups long before I suspected the presence of a pair of idiochromosomes in this genus.

The usual conclusion follows that spermatozoa containing the large idiochromosome produce females and those containing the small one produce males. It is equally clear that the *s*-chromosome, though unpaired and hence a heterotropic chromosome in behavior, is not physiologically comparable to an odd or "accessory" chromosome of the usual type.

The numerical relations between Types A and B are interesting. Since in maturation the *s*-chromosome couples more frequently with the small idiochromosome (which is confined to the male) we should expect to find the *s*-chromosome in a majority of the males and in a minority of the females; and such is indeed the case in the 12 individuals that have been examined. Of the seven males, five are of Type A and two of Type B — a ratio that happens to be nearly identical with that shown in the coupling. Of the five females on the other hand, only one is of Type A (with three small chromosomes, Fig. 1, *c*),[1] while four are of Type B (Fig. 1, *f*). The number of individuals is of course too small to give an accurate result; but as far as they go the facts are in conformity with the expectation created by the mode of coupling in the spermatogenesis.[2]

BANASA.

The remarkable relations observed in *Metapodius terminalis* probably give the explanation of those I formerly described in *Banasa calva*, though I am not yet in a position to prove this positively. I have now new material of this genus from individuals ranging from New England to Arizona, and comprising both of the more frequent species, *B. calva* and *B. dimidiata*.[3] All the new material of *calva* differs from the Long Island material that I formerly described in the *absence of the small unpaired or heterotropic chromosome*, though in every other detail they are identical. To facilitate the comparison I give three new figures from the Long Island material (which, as above stated, includes only two slides from the Paulmier collection). The first division here always shows 15 chromosomes (Fig. 2, *a*) of which two, the unpaired chromosome and the small idiochromosome, are much smaller than the others. Owing to the passage of the unpaired chromosome to one pole without division in the first maturation division the secondary spermatocytes are of two types, showing respectively 14 and 13 chromosomes (Figs. 2, *b*, 2, *c*) — a relation shown with perfect clearness in a large number of cells. In all my new material on the other hand (from New York, Ohio, Colorado and New Mexico) the chromosome groups are exactly similar to those of the Long Island form except that the small unpaired chromosome is missing. The first division accordingly always shows 14 chromosomes instead of 15, of which one (the small idiochromosome) is smaller than the others (Fig. 2, *d*). The second division always shows 13 chromosomes, (Fig. 2, *e*) of which one is a typical idiochromosome-bivalent; and in the ensuing division all the spermatids receive 13 chromosomes, half receiving the small idiochromosome and half the large. Both the spermatogonial and the ovarian groups accordingly show 26 chromosomes, the small idiochromosome being present in the male only (Fig. 2, *f*). In every respect, therefore, these individuals show the typical pentatomid relations, and agree with Type B of *Metapodius*.

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[1] This individual differs from all the others in having 22 instead of 20 large chromosomes, or 25 in all. I have found a similar variation in the number of larger chromosomes in different individuals of two other species of the genus (*M. femoratus* and *M. granulosus*) as will be described hereafter. These variations appear to have no constant relation to the presence or absence of the *s*-chromosome and hence do not affect the questions here under consideration.

[2] Besides the two types of males and females described above we should expect to find a third type in each sex containing two *m*-chromosomes and two *s*-chromosomes. Such forms have not yet come under my observation, and it is possible that gametes containing both these forms of chromosomes are infertile towards each other.

[3] I am indebted to the well known hemipterist Mr. E. P. Van Duzee, of Buffalo, for the identification of these and many other species.

Banasa dimidiata agrees essentially with this except that to my astonishment the number of chromosomes was found to be much smaller, namely, in the spermatogonia 16 (Fig. 2, *j*), in the first division nine (Fig. 2, *g*), and in the second eight (Fig. 2, *h*). It is noteworthy that these two species, which are so closely similar as sometimes to have been confused by systematists, should differ so widely in the number of chromosomes.

The difference between the material from Long Island, labeled "*Banasa calva*," and my own at first led of course to the suspicion that an erroneous identification was at fault; and this is indeed possible since the Paulmier slides were not accompanied by the original specimens. But the exact similarity of the two forms in every respect apart from the unpaired chromosome, and my failure to find any other similar form in an examination of nearly all the species of Pentatomidæ that might be confused with this species, leads me to believe that the case of *Banasa* *calva* is probably similar to that of *Metapodius terminalis*, the Long Island form being of one type (corresponding to Type A of *Metapodius*, with an unpaired *s*-chromosome) and the others of the other type (corresponding to Type B of *Metapodius*). In *Metapodius* the two types occur side by side in the same locality ;

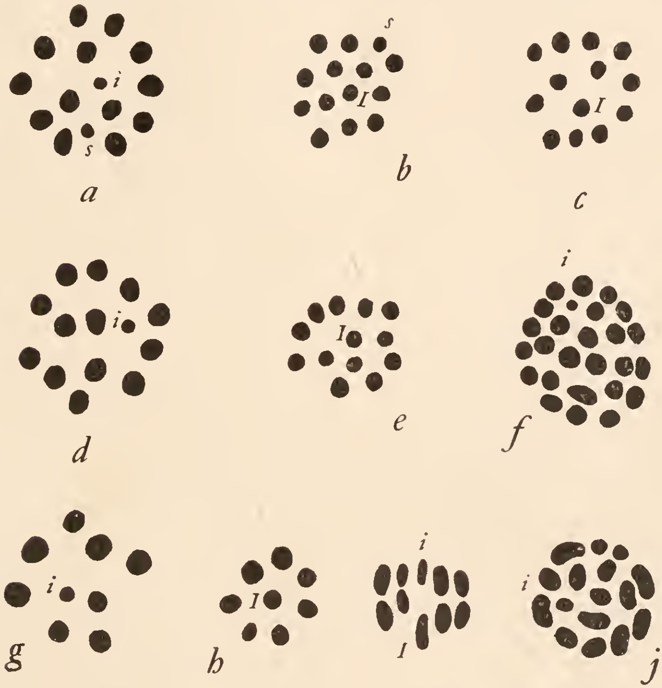

FIG. 2. *Banasa.* (*a–f*, *B. calva*, *g–j*, *B. dimidiata.*) *a*, Long Island form (Paulmier), first spermatocyte-division ; *b*, 14-chromosome type of second division ; *c*, 13-chromosome type, from the same cyst ; *d*, western form (Colorado), first spermatocyte-division ; *e*, second division of same ; *f*, spermatogonial division ; *g*, *B. dimidiata*, first division ; *h*, second division, polar view ; *i*, side view of same ; *j*, spermatogonial group. (In *a* it is impossible to distinguish between the *s*-chromosome and the small idiochromosome.)

and the same should be true of the Long Island *Banasa calva* if the two cases are really alike. But this question can only be settled with additional material. All the individuals from western New York (Buffalo) and further west are of Type B. It therefore seems not improbable that one of the types has been lost in the western forms, or conversely, that Type A has been added to B in Long Island, perhaps in a local colony or variety.

COMMENT.

The strong support lent by the foregoing facts to the general theory of the individuality — or, as I should prefer to say, the genetic identity — of the chromosomes is so obvious as hardly to require comment. I will here only call attention to the interest of the coupling of the *s*-chromosome with one of the idiochromosomes in *Metapodius*. This phenomenon is doubtless comparable in a general way to the coupling of the true odd or "accessory" chromosome with one of the ordinary bivalents first briefly recorded by Sinéty[1] in the Phasmidæ, and carefully studied in several of the grasshoppers (in some of which the facts are more complicated) by McClung[2] who has given an interesting discussion of the subject. I have observed chromosome-couplings in four families of the Hemiptera heteroptera and believe the phenomenon will be found to be of wide occurrence in the insects, and perhaps in other animals. It seems well within the bounds of possibility that such chromosome-couplings may give the physical basis of certain forms of correlation in heredity. If the chromosomes embody the primary factors of heredity (the working hypothesis upon which I am proceeding in these studies), it must no doubt be assumed that each chromosome contains the determinants of many characters; and the association of such determinants in the same chromosome may imply the constant correlation of the corresponding characters in heredity. But in addition to this, certain correlations, such as are observed in some forms of hybrids, might also be a result of a more or less pronounced tendency of certain chromosomes to cohere in a definite way, so as to be more frequently or even invariably associated in the germ-cells. In the case of *Metapodius* such a tendency is shown in the more frequent coupling of the *s*-chromosome with the small idiochromosome, which leads to its more frequent passage to the male-producing pole, and hence to its more frequent appearance in the male. This reminds us of certain crosses of Lepidoptera observed by Standfuss,[1] and more recently by Doncaster and Raynor,[1] in which there is a tendency for a particular set of specific or varietal characters to appear more frequently in one sex than the other. Thus, in *Abraxas*, as reported by the last named observers, after crossing the original form (*A. grossulariata*) with an albinistic variety (*lacticolor*) to which it is dominant, the cross ♂ DR × ♀ RR gives both sexes of both forms, but the reverse cross ♀ DR × ♂ RR results in a sharp separation of the sexes of the two forms, all the resulting males being DR and the females RR. This, as the authors show, may be explained by the assumptions, first, that the sex borne by the egg is uniformly dominant (as appears to be the case in the Hemiptera) and second that the dominant somatic character (*i. e.*, the *grossulariata* pigmentation) uniformly couples with the male character in the egg while in the spermatozoon no coupling occurs. Such a chromosome-coupling as that observed in *Metapodius terminalis* gives a very definite basis for the possible explanation of couplings of the sexual characters with specific or varietal ones; and it seems possible that we may in this direction find a means of testing decisively the whole chromosome-theory of heredity. In the case of *Metapodius* I have not thus far been able to find any constant differences between individuals of Types A and B; but only the external characters are available for examination. I hope hereafter to examine this question more thoroughly, both in *Metapodius* and in *Banasa*.

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[1] *La Cellule*, XIX., 1901–1902.

[2] BIOL. BULL., IX., 5, October, 1905.

ZOÖLOGICAL LABORATORY, COLUMBIA UNIVERSITY,
February 4, 1907.

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[1] See Castle, *Bull. Mus. Comp. Zool.*, XL., 4, 1903.

[2] *Proc. Zool. Soc. London*, June 7, 1906.

Bio Bull V12 (1907), 314-345

SPAWNING BEHAVIOR AND SEXUAL DIMORPHISM IN FUNDULUS HETEROCLITUS AND ALLIED FISH.

(Contributions from the Zoölogical Laboratories of the University of Michigan. No. 108.)

H. H. NEWMAN.

Introduction.

Of recent years much stress has been laid upon the structural basis of behavior, especially among the lower organisms. Among the higher animals, on the other hand it has long been understood that function and structure are simply dynamic and static phases of the same thing. There would be little excuse for the present paper, then, unless it should serve to show that these ideas of structure and function — sexual dimorphism and spawning behavior — have a far wider application than has commonly been supposed. It will be shown that even minute, temporary structures, that have previously escaped the eye of the investigator, are as truly adaptations for spawning as are the more obvious secondary sexual characters, such as differences in the sizes of fins, in color pattern, in body form, etc.

My attention was first called to this subject by chance. One day early in the summer of 1906, while engaged in cross-breeding species of *Fundulus* at the Marine Biological Laboratory of Woods Hole, I was fortunate enough to observe the spawning act in the species *Fundulus heteroclitus.* These fish were spawning in a small aquarium and in a good light so that the entire process could be observed in minute detail without difficulty. Afterwards I was fortunate in being able to observe the spawning of *Cyprinodon* under equally favorable conditions. These observations led to a closer study of the behavior of these species and to a consideration of their sexual dimorphism as the structural basis of this behavior. The other two available species of *Fundulus*, *F. majalis* and *F. diaphanus* were then brought in for purposes of comparison.

The wash drawings reproduced in Plates XXVII. and XXVIII. were made under my direction by Miss Ella Weeks of the State Agricultural College of Kansas. I take this opportunity of expressing my appreciation of the quality of her work and my indebtedness to her for her service. I also wish to express my thanks to Professor Jacob Reighard for his helpful suggestions and criticism.

The plan of the paper is to treat each species separately, to give a summary of the main points, and to conclude with a general discussion of the origin and significance of the structure and behavior described.

The four species dealt with all belong to the family Pœciliidæ (the killifishes). The following statements referring to sexual dimorphism in this family are quoted:

> "Sexes usually unlike, the fins being largest in the males, but in some species the females are much larger in size. Many of the species are ovoviviparous, the young well developed at time of birth. In these species the sexes are very unlike, the anal fin of the male being developed into an intromittent organ."—Jordan and Evermann, "Fishes of North America," p. 631.
>
> "In many species the sexes are dissimilar, the female being larger and less brilliantly colored, with smaller fins."—"Cambridge Natural History."

I have been unable to find anything in the literature concerning the spawning behavior of these species.

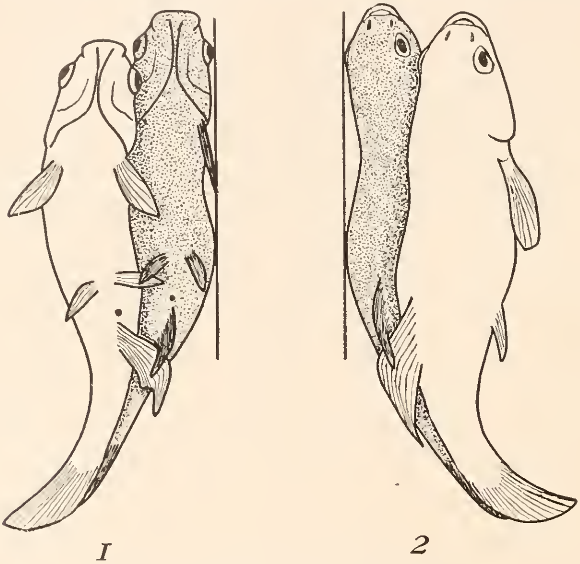
For purposes of clearness it seems best to present the facts on spawning behavior before those on sexual dimorphism, since behavior throws so much light on the significance of structures.

FUNDULUS HETEROCLITUS (Linn.).

(Common Killifish; Mudfish; Cobbler; Mud Dabbler; Mummichog.)

Spawning Behavior.

The spawning act proper was the first to gain my attention and it is hardly likely that I should have noticed it had I not been slightly familiar with Professor Jacob Reighard's unpublished observations on the spawning habits of certain inland species. In spawning the male clasps the female firmly around the slender portion of the body just back of the dorsal and anal fins, using as claspers his large, strong anal and dorsal fins. The two fins of the male slip in under the homologous fins of the female, which are raised up to admit them, and nearly surround the body of his mate. The ventral fins of adjacent sides are also locked in similar fashion. The female is usually forced against some perpendicular solid object such as a stone, a mass of seaweed or the glass sides of the aquarium — most frequently the latter when in captivity. When the female is thus seized by a male she suddenly assumes a characteristic attitude, the whole body, when observed from above having the conformation of a somewhat flattened S, the head being pressed against the solid, that part of the body just back of the head being bowed outwards and not touching the solid surface, the region of the abdomen being again against the solid, and finally the whole tail region being free from the solid and bent sharply away from it and slightly upwards (see Text Plate I.). In this rigid position she is supported from the bottom by means of her anal fin, which



Text Plate I. Showing the spawning attitude of male and female Fundulus heteroclitus, the female darkened.

Fig. 1. Ventral view, showing the position of anal and ventral fins.

Fig. 2. Dorsal view, showing the position of dorsal fins. This view is a half side view. The straight line indicates the points of contact with a solid substance such as the glass sides of the aquarium. ½ natural size.

extends nearly at right angles from the belly and is thick and stiff and well adapted for supporting the weight of the two bodies and often becomes much inflamed by frequent contact with the bottom. The male, clasping the female firmly as he does, holds his entire body against hers for from one to two seconds. While in this position a quivering vibration of the posterior half of the bodies of both fish occurs, during which eggs and sperm are extruded in very intimate contact with each other. Whether this vibratory movement is initiated by one or both sexes I cannot say for certain, but I am inclined to believe that the female only is responsible, the male simply remaining passive and taking up the vibration from the female. My reasons for believing that such is the case are: first, the vibratory rhythm of the two fish spawning together is always in perfect unison, which would hardly be the case when two fish of very different sizes clasp; second, I have often seen females assuming the spawning attitude and going through the vibratory movement when there were no males in the vicinity.

This spawning act is highly adaptive in several respects:

1. The vibratory movement serves to extrude ripe eggs and ripe sperm at the same instant. It is only in this way that the sexual products can be extruded as is shown by the fact that the females are forced to adopt this method of relieving themselves when there are no males present.

2. The position assumed by the two sexes is such that eggs and sperm are extruded in very close proximity and could scarcely fail to come in contact. Chances of failure are minimized by the fact that on the one hand the milt is shot out with considerable force directly at the eggs as they are extruded, and on the other the eggs exert a strong chemotactic influence upon the sperm, which I demonstrated by the following experiment. A freshly stripped egg was put on a glass slide with a few drops of water and then a drop of sperm was put in the water at a distance of half an inch. In a very short time the egg was surrounded by a dense cloud of sperm and inside of two minutes all of the sperm was seen to be gathered about the egg. In addition to this the surface of the egg is very glutinous and probably holds all sperm that comes in contact with it.

3. The position of the anal fin of the female is such that it supports the weight of both fish, the distal end of the fin being in contact with the bottom which under natural conditions is usually composed of soft mud. Since this fin receives the vibratory movement of the body it necessarily stirs up a considerable amount of mud and also makes a shallow depression. The eggs, as soon as they are extruded, fall to the bottom and either settle in the small hole or are at least partially concealed by the settling mud. That some such protection is necessary is shown by the fact that the fish themselves are very fond of their own eggs, devouring them eagerly when they can be seen. This curious type of cannibalism was observed repeatedly in aquaria where the bottom was free from mud. The females were the chief offenders in this respect although young males occasionally devoured the freshly extruded eggs. It was a very common sight to see other females rush up and seize the eggs as soon as they were extruded. Frequently, however, females were observed to turn and devour their own eggs. This destruction of eggs probably occurs only to a very limited extent under normal conditions for the reasons cited above.

Courtship, Rivalry and Display.

As might be expected, the male takes the more active and aggressive part in courtship but the female frequently displays herself in such a way and assumes such postures as are calculated to attract the male. Females laden with very ripe eggs frequently display themselves by turning on their sides near the bottom, and spurning the latter with their tails, thus causing their silvery white bellies to flash in the light. This, I noticed, seldom failed to attract the males if there were any of the latter about. This curious flashing movement is by no means restricted to the females of this species, but was observed occasionally in the males of the same species, in both sexes of *Fundulus majalis*, and in several other marine species. It was, however, especially noticeable in actively spawning females and seems to be of undoubted service in attracting males.

When both sexes are at the height of their sexual activity there is little that could be termed courtship. The females are burdened with their great masses of eggs, that must distend their adbomens uncomfortably, and are eager to get rid of their burdens. This can most easily be accomplished by contact with the males, but may be and frequently is accomplished without the latter. The females usually, however, retire to the bottom and place themselves in contact with some solid object, sometimes only the snout being actually in contact, thus assuming a position in which they may be most readily clasped by the male. Whenever a male sees a female in this position he loses no time in spawning with her. If a female after taking up the position just described is not joined by a male she is very apt to relieve herself by assuming the S-shaped posture characteristic of the spawning act and by vibrating her body just as she would if clasped by the male. The eggs are thus plentifully extruded, but if no males are present, they are not and probably never can be fertilized. If, however, any males are in the vicinity they are always attracted by the vibratory movement and dart toward the source of vibration.

Courtship, if such by courtesy it may be called, occurs shortly before the sexual climax. Fish in this condition swim about comparatively quietly in pairs, the female above and the male just below and slightly back of her. This position enables him to see her more readily and at the same time to guide her about by gently butting her on one side or the other with the top or sides of his head. I have observed very many pairs swimming about in this way for considerable periods of time. Gradually the male becomes more excited in his movements and the preliminary courtship merges into spawning behavior proper. At first the attempts at spawning on the part of the male are apt to consist merely of efforts to corner the female and to induce her to seek some retired spot at or near the bottom. To accomplish this he rises from beneath her and butts her downwards with the sides of his head. If he succeeds in driving her into a suitable place he attempts to spawn with her, the first few trials lacking the vigor characteristic of ruling males. Sexual excitement increases rapidly so that before many minutes have passed the male is apt to be seen spawning promiscuously with any female that he encounters. This transition from courtship to spawning was observed both in aquaria and under natural conditions. In the open, excited males were often seen to be in rapid pursuit of the females, succeeding occasionally in cornering and clasping them. The pursuit of the males was often so impetuous that the females were entirely frightened away. The coyness on the part of the females acts as an excitant on the males.

Rivalry among the males is very keen and as a rule those whose "spawning plumage" is most brilliant succeed in driving away all competitors. I have observed under normal conditions that a certain male, always the most brilliantly colored one in the neighborhood, seems to control the situation, driving away all males that attempt to encroach upon his territory. The size of the male seems to be a much less important factor in determining his success than is the degree of sexual maturity as indicated by the brilliance of his coloration, for I have often seen a male of comparatively small size put to rout several others of twice or three times his size, such is the impetuosity of his attack. I was able to observe this rivalry to better advantage in aquaria where it was possible to identify the various individuals and thus to keep an accurate record of the success or failure of each male. The following record was made on June 20, 1906.

A fresh lot of *F. heteroclitus* was placed in the aquarium early in the morning. At about ten o'clock in the forenoon they were observed to be actively spawning. About twenty females were spawning on the bottom of the aquarium on the side away from the light. Seven males seemed ready to take part in the process, but one very large male, more brilliantly colored than the rest, continually drove away all other males that attempted to spawn. So solicitous was he about driving off his competitors that he could scarcely attend to all of the females that were ready to spawn. While the ruling male was engaged in clasping one female another male in a remote portion of the aquarium would occasionally succeed in spawning with another female. Whenever such an occurrence was observed by the ruling male, he always gave chase and invariably routed the intruder. This male was so much larger and more vigorous than any of the others that none dared to dispute his authority to the extent of offering battle. For purposes of experiment I removed this male from the aquarium and put him in a smaller vessel by himself, being careful to keep him out of the strong light. When thus removed from the sexual environment, he almost immediately underwent a very marked change in appearance, becoming decidedly lighter in color, and inside of ten minutes losing all of the steely blue glint that is so characteristic of sexual excitement. Putting a dark colored fish in a strong light causes a similar lightening of color, but that the change in question was not due to light seems certain for all strong light was excluded. As soon as this ruling male had been removed the other males usurped his prerogative and a struggle for supremacy immediately ensued. The combat between the six remaining active males was for a time very evenly waged, since there was no great disparity in size, but after about ten minutes a single male, and he not the largest, had gained supremacy and had succeeded in driving away his rivals whenever they approached. Occasionally one of the outsiders plucked up sufficient courage to challenge the ruling male and a combat ensued. The males fight with their heads and mouths, butting one another fiercely and accasionally locking jaws and struggling like dogs. When a male wishes to challenge he approaches rather cautiously, body trembling with excitement and all fins extended to the utmost, presenting as formidable an aspect as possible. The male thus challenged adopts a similar attitude and rushes at his foe with alacrity. Curiously enough the male that has once gained supremacy always emerges victorious from these contests, and the defeated male retires into hiding until he has regained sufficient courage to challenge again. After about half an hour the large male that had been removed to another vessel was returned to the aquarium. At first he seemed to take no interest in the spawning activity going on about him, but gradually he aroused himself and made an occasional half-hearted attempt to clasp a female that came near him and advertized by her attitude her desire to spawn, but he was always rudely interrupted and put to rout by the new ruling male, which although of very much smaller size, attacked with such vigor that his much bulkier opponent was forced to retire. By degrees, however, the large male increased in vigor, at the same time growing darker and reassuming the blue glint that he had so quickly lost. In about twenty minutes he was as dark and as brilliant as before and had succeeded in ousting the usurper from his domain, although not without repeated struggles in which his victory became more decisive each time. The next day the same male was in control, but on the following day another male of medium size had acquired supremacy. It is probable that the period of sexual climax is of short duration, not exceeding three or four days. A male at the very height of his sexual activity is afraid of nothing and is practically invincible.

This account will serve as a sample of the scenes observed repeatedly in the aquaria and in natural conditions during the months of June and July. The last recorded observation of spawning in this species was taken on July 7, although ripe males and females were found for at least two weeks later.

This account of the spawning behavior of *Fundulus heteroclitus* may well be concluded with an account of a few more experiments and some additional isolated observations.

Experiment 1. — A considerable number of actively spawning males and females were separated into two aquaria, the males in one and the females in another. Inside of about fifteen minutes the males had all become nearly as pale as the females and spent their time in wandering about uneasily as though seeking for a place of escape. The females, on the other hand, seemed to be very little affected by the absence of the males but went on extruding eggs as freely as if the males had been present. It is probable that the initial stimulus to egg extrusion given by the males lasted some time after the removal of the latter. The eggs were always eagerly devoured either by the female that laid them or by another that rushed up and siezed them before she could turn around. It is hardly probable that eggs are so eagerly devoured in the open, as the food supply is not restricted as in an aquarium.

Experiment 2. — An aquarium was prepared with mud and stones on the bottom to approximate natural conditions, and in it were placed five spawning fish of each sex. These fish, instead of appearing to enjoy their new surroundings, lost all interest in spawning and spent all of their time in exploring their environment. In the meantime the males lost all of their "spawning plumage" and became decidedly pale. The females too seemed to have forgotten about spawning in their anxiety to become familiar with the new neighborhood. On the following day the fish were still uninterested in spawning and I concluded that they had passed the sexual climax while they had been busy with their explorations.

Experiment 3. — I put several males that had been isolated for several days into an aquarium containing only females. These had been extruding eggs at intervals, but as soon as the males appeared, they seemed to become excited and immediately began to take up spawning attitudes and to extrude eggs in much increased amounts. It seems certain that the presence of the male, even when the latter refrains, as in this case, from any participation in the spawning act, exercises an exciting influence upon the female. The stimulus is probably a visual one, for the appearance of the male is very characteristic.

Additional observations :

1. Occasionally pairs were observed to come together and spawn in open water without being in contact with any solid. It was also not unusual to see them spawning against the bottom instead of against some more or less perpendicular object.

2. On three occasions I observed a female following a male around and apparently endeavoring to incite him to spawn with her by bumping him and placing her body in contact with his. On one occasion she succeeded in inciting him to clasp her for an instant. This assumption of the initiative on the part of the female struck me as being decidedly abnormal and may have been a perversion of instinct, due to confinement.

3. I observed that females that were being guided about by the males occasionally seemed to resent this infringement upon their liberty and engaged in a somewhat mild form of contest with the males, returning their butting in kind. The male, however, invariably seemed to have his way in the end.

4. On June 27, I observed *F. heteroclitus* spawning in the Eel Pond in the shadow of a boat and in about eighteen inches of water. The males would chase females out beyond the shadow but usually returned quickly to the shade. I have noticed repeatedly that the fish prefer the darker places for spawning.

5. After watching the spawning of this species in the open, I believe that large males, when at the height of their sexual period, control considerable areas in the Eel Pond and elsewhere. Although an active male may pursue a female or another male for considerable distances he soon returns to the neighborhood over which he seems to exercise authority. This phenomenon is by no means unusual in fish.

Sexual Dimorphism.

The following passages, refering to sexual dimorphism, are selected from Jordan and Evermann's systematic account:

> ". . . fins moderate, the dorsal inserted in males midway between snout and tip of caudal; in females farther back; oviduct attached to anterior ray of anal fin for one-half to two-thirds its length; . . . Coloration in males dark dull green, the belly more or less orange yellow; sides with numerous quite narrow, ill-defined silvery bars made up of silvery spots, most distinct posteriorly; besides these are numerous conspicuous white or yellow spots, irregularly scattered; vertical fins dark with numerous small round pale spots; dorsal often with a blackish spot on its last ray; anal and ventrals yellow antertiorly; under side of head yellow; young males with alternate bars of dark and silvery, the former becoming in time the ground color, the dorsal ocellus more distinct. Females nearly plain olivaceous, lighter below, without spots or bars, the scales finely punctate; sides often with about fifteen dark crossbars or shades. Young, especially young females, with more or less distinct dark cross bands; these always present in the very young, in females narrower than the interspaces, in males much broader and less numerous."

This description, while accurate enough so far as it goes, needs to be supplemented with regard to certain details. It also fails to take into consideration the fact that there are marked seasonal changes not only in color but in the actual size of certain parts such as belly and fins, and in the production of certain temporary organs in the male.

First of all I would like to supplement Jordan and Evermann's account and to call attention to certain points. Then I shall be in a position to discuss the seasonal changes.

The description of the male is fairly accurate for one out of the breeding season. I wish, however, to call attention to the relatively large size of the dorsal and anal fins of the male as compared with those of the female (see Plate XXVII., Figs. 1 and 2).

The enormous difference in the fin coloration of the two sexes should also be emphasized, those of the female being almost devoid of pigment while those of the male, although more deeply colored in the spawning season, are always markedly pigmented, the pigment being laid down is such a way as to produce a mottled pattern. The posterior and proximal half of the dorsal is, however, always much darker than any other area on the fins and is the equivalent of the much more distinct spot seen on the dorsal of male *Fundulus majalis*. There is also a marked difference in the shape and in the strength or stiffness of these fins in the two sexes. In the male there is a pronounced posterior prolongation of both fins, especially the anal. These fins are stronger and better provided with muscle in the male than in the female and hence are better fitted for clasping organs.

Discussion of the Seasonal Changes.

The seasonal changes may be classified as follows:

In the female:

1. Paling of the general body coloration.

2. Distension of the abdomen with eggs and consequent lessened activity.

3. Thickening and inflammation of the anal fin.

In the male:

1. Intensification of pigmentation in definite regions.

2. Acquisition of a steely blue gleam in the scales of certain regions. (1 and 2 are spoken of collectively as "spawning plumage.")

3. Development of certain temporary organs on the scales that I have chosen to call "contact organs."

1. The paling of the general body coloration in the female and the intensification of pigmentation in the male might be attributed to the opposite metabolic conditions prevailing in the two sexes at this period. The female, having to sacrifice so much of her vitality for egg production, must have a lowered somatic metabolism, the index of which is the diminished production of pigment. The male, on the other hand, seems to have much excess vitality, since the production of sperm is far less taxing on somatic vitality than is the production of eggs. The deposition of pigment here

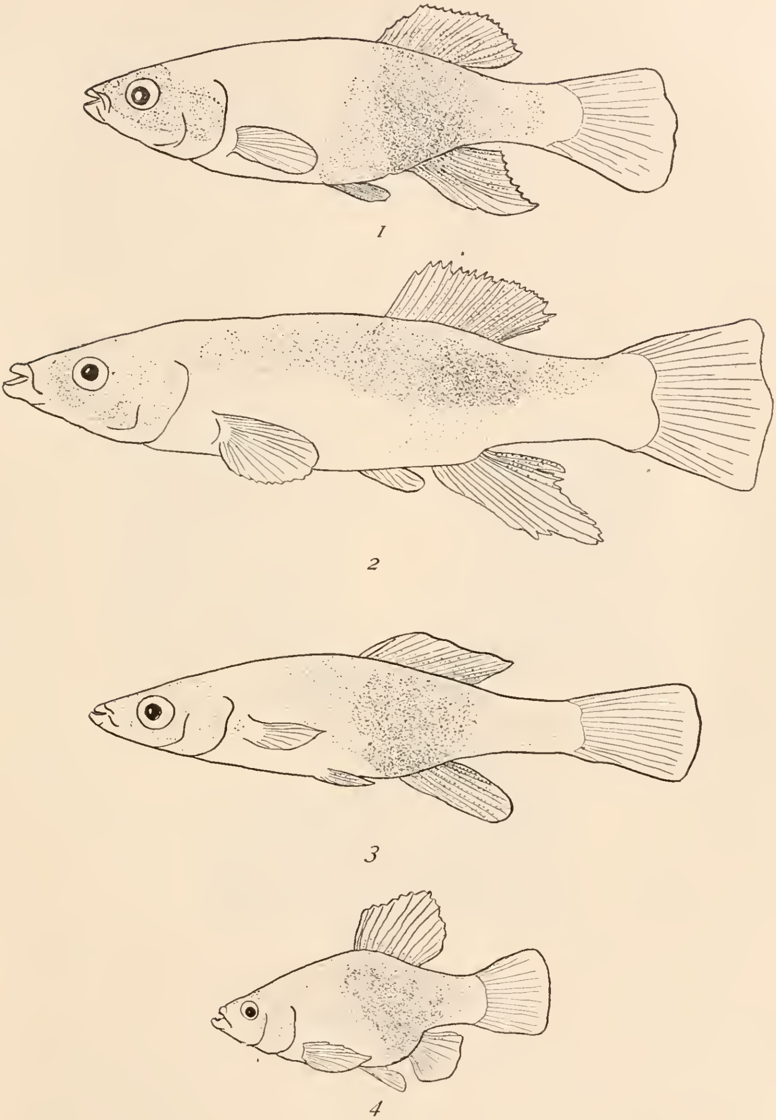
is probably an index of a very rapid metabolism that expresses itself in other ways such as greater activity, greater courage, and in the production of excrescences, etc.

2. The thickening of the anal fin in the female may be partially a phenomenon of inflammation produced by irritation. The sources of irritation are twofold. In the first place the tip of the fin is rubbed violently against the bottom during the spawning act. In the second place the frequent expulsion of eggs through the tubular extension of the oviduct that runs down the posterior ray of the fin, probably causes inflammation in this and adjacent parts. The thickening of the fin gives a firmer support for the spawning pair than would the fin in its usual condition, and in addition stirs up the mud more effectively as has been shown.

3. The steely blue gleam so characteristic of spawning males, reminds one of iridescence but is not of the same character. The blue color is probably due to a combination of chromatophores and iridicytes. The former are extremely extensible in that the pigment is capable of flowing out over a wide area through slender, branching canals. The latter are extremely minute prismatic crystaline bodies that serve the purpose of refracting the light. They evidently lie above the chromatophores which furnish the absorbing background. In some way the colors at the red end of the spectrum are absorbed by the melanin and the combined colors of the violet end of the spectrum are reflected as the steely blue gleam. The extension and contraction of the melanophores seems to be a reflex closely associated with sexual excitement, and may be considered as a sort of involuntary flush.

4. The structures that I have chosen to designate as contact organs, occur as finger-like processes on the margins of all the scales in certain regions, and upon the fins that are used in clasping. The appearance of these processes is well shown in the photographs reproduced in Text Plate III.

It is of interest to note that contact organs occur only on actively spawning males and only upon those parts that are in contact with the female during the spawning act and upon the top and sides of the head, parts that are used for butting the female in courtship and one another when fighting. The distribution of the contact organs in the four species studied is represented in

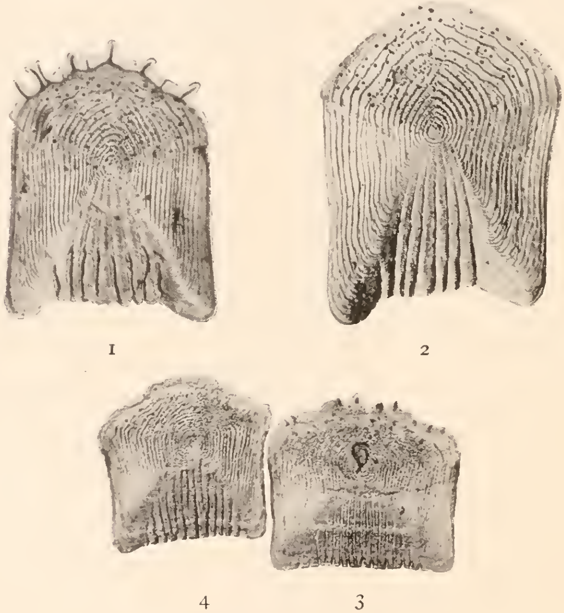


TEXT PLATE II.

Showing the distribution of contact organs in males of the four species. The stippled areas represent regions supplied with contact organs. Where the stippling is heaviest the contact organs are most numerous and best developed. The dots on the fins represent contact organs.

1. *Fundulus heteroclitus.*
2. *F. majalis.*
3. *F. diaphanus.*
4. *Cyprinodon variegatus.*

Text Plate II. It is also interesting to note that the best developed and most frequent contact organs occur where the pressure in spawning must be the greatest, namely between the dorsal and anal fins and on these fins at or near their bases. In each case the maximum distribution seen in the males collected is shown in the figure. The stippling is closer in regions where the contact organs are thickest and best developed, and more open where the latter are more scattering and less well developed. No attempt has been made in these diagrams to represent actual numbers or sizes.



Text Plate III.

Showing scales with contact organs, × 12.

1. Scale from the side of a large male *F. majalis*.

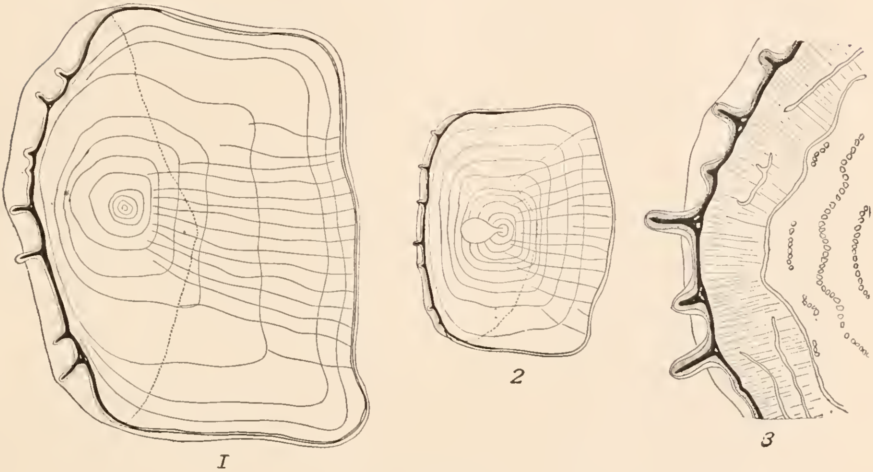
2. Scale from a corresponding part of the body of a large female *F. majalis*.

3. Scale from the lateral line region between the dorsal and anal fins of a spawning male *Cyprinodon variegatus*.

4. Scale from a similar location of a spawning female *Cyprinodon variegatus*.

An examination of a considerable number of males reveals the fact that only those in spawning plumage possess contact organs, while some of these possess them only on the top or sides of the head. It is my belief, although I am not at present in possession of any direct evidence on the subject, that the organs are developed antero-posteriorly, either in response to a fixed law of development or because there is an earlier need for these structures on the cheeks and on the top of the head, since these parts are used in the preliminary courtship, coming into frequent contact as they do with the body of the female.

The contact organs are practically alike in all of the species studied, although they vary slightly in form and in size relative to that of the scale on which they occur. They are best developed in *Fundulus majalis*, but are simply larger here than in the species under consideration. Photographs and camera drawings (see Text Plates III. and IV.) show clearly the appearance of the contact organs in typical scales taken from males of the four species.



TEXT PLATE IV.

Camera drawings of typical scales from the region between the dorsal and anal fins of spawning males.

1. *Fundulus heteroclitus.*

2. *F. diaphanus.*

3. Enlarged detail drawing of a portion of the exposed margin of a scale taken from the side of a male *F. majalis.* The black region represents the horny margin of the growing region of the scale that is prolonged into spikes that support the contact organs. The stippled area represents the dermis. The clear outside area represents the epidermis. The striated portion is the non-calcified portion of the scale.

They consist first of a core of horny material or sclerified connective tissue (see Text Plate IV., Fig. 3), that arises like a sharp spike from the free growing edge of the scale. This skeletal support is represented in black in the drawing although in life it is nearly transparent. Various stages in the development of the spikes may be seen both in the detail and in the general drawings. They first appear as slight outwardly directed folds of the edge of the growing region of the scale, and gradually assume the spike-like form. Outside of this horny support there is a fairly thick layer of dermis, represented in the detail drawing in stippling. The histological characters of this layer I have been unable to make out in the formalin preserved specimens that have been my only resource in the present paper. Outside of the dermis there is a thin layer of epidermis that is often found worn off at the tips of the papillæ, allowing the horny spike to protrude.

The contact organs do not lie flat against the body of the fish, but stand out at an angle of about thirty degrees, so that they can readily be seen in profile with the naked eye. This attitude is decidedly advantageous for giving a rough surface or for a sensory function. Probably the former function is the principal one, although I am not sure that the latter function is not subserved. If the contact organs should prove to be sensory we can understand how their stimulation by the vibration of the female during the spawning act might account for the extrusion of sperm on the part of the male. These points have not been made out on account of the lack of histologically fixed material, but a study of the histology and function of these organs will furnish material for a more special paper.

It should be stated that the contact organs are possibly related to the so-called "pearl organs" found in other species of fish. Their structure, however, is entirely different in that they are chiefly dermal in origin and possess the horny spike-like support, while pearl organs are little more than epidermal callouses.

The resemblance of the contact organs to the teeth on the margin of ctenoid scales will probably strike the reader. It has occurred to me that here we have the origin of the ctenoid type of scale. Ctenoid scales are found on the most highly specialized of our Teleosts but are described as being absent in more primitive families such as the one with which we are dealing. Is it not possible that we have in this family ctenoid scales developed as mere temporary structures, used only by the males during the spawning season? If we admit the possibility of this condition we can see how such structures might become permanent, be produced in both sexes and subserve another function.

Fundulus Majalis.

(Killifish; Mayfish; Rockfish.)

Sexual Dimorphism.

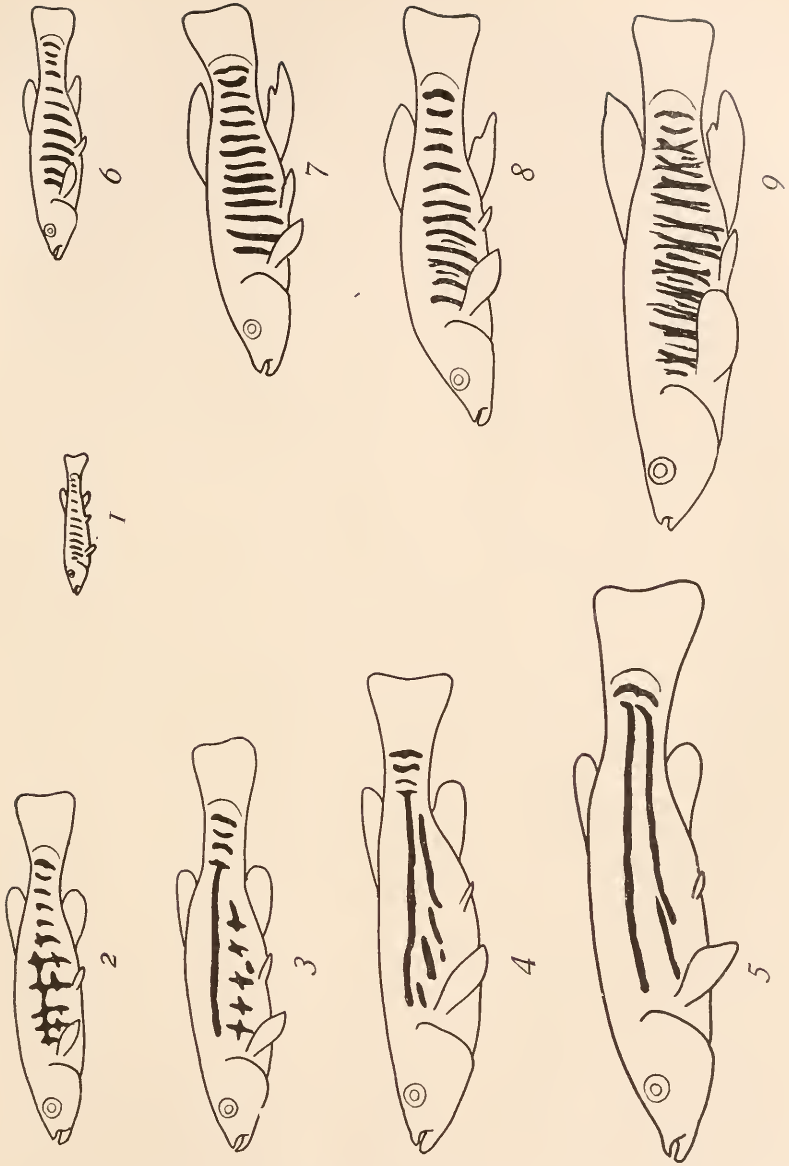
The following passages, referring to sexual dimorphism, are selected from Jordan and Evermann's account:

> ". . . anal fin very high in males, moderate in females; ventrals long in the males, reaching past front of anal; . . . Males dark olivaceous above; sides silvery or somewhat golden, with about a dozen broad transverse bars of the color of the back; posterior part of the dorsal fin with a black patch; fins yellowish or pale. Females olivaceous above, white below, a narrow black longitudinal stripe along sides about on the level of the eye and as wide as the pupil; below this two similar black stripes anteriorly and one posteriorly, the upper one being interrupted; one or two black bars at the base of caudal. Females usually larger than the males. A large male of this species, in high coloration, taken at Beaufort, N. C., showed the following colors in life: Back olive, sides and belly bright salmon yellow; lower fins clear yellow; pectorals and anals with some dusky; posterior edge of caudal dark; dorsal nearly all black, a large black ocellated spot on the last rays; opercles and under parts of head with an inky suffusion; cheeks, top of head, and mouth bronze yellow; sides with about eighteen narrow, dusky vertical bars."

The "high coloration" referred to in the Beaufort male is undoubtedly the "spawning plumage" and, although a somewhat more highly colored condition is described than I have observed in any male at Woods Hole, the account is probably entirely accurate for the species in more southern waters. The males that I have observed (see Plate XXVII., 4) have the back and the upper part of the sides almost black instead of olive as described; lower part of the sides and belly orange or golden yellow instead of salmon yellow; dorsal, anal and caudal fins golden with a tendency toward dusky, the dorsal being only a trifle darker than the other fins instead of being black as described above;

cheeks top of head, etc., heavily shaded with a bluish-black coloration, reminding one of the blue glint of the male *F. heteroclitus* instead of bronze yellow as described. The black spot on the posterior rays of the dorsal may, as in the illustration (Plate XXVII., 4), be composed of a series of spots arranged in a sort of circle. This is more apt to be the case in very large specimens.

The color pattern of both sexes is gradually modified during the lifetime of the individual and all stages in the production of the complete adult pattern are readily found. The young of both sexes are always cross barred somewhat like the adult male, but the bars are less numerous, numbering seven to ten as compared with from fourteen to twenty in adults. The increase in number of bars takes place by means of a longitudinal splitting of individual bars and by the appearance of small new bars between the old ones (see Text Plate V., Figs. 8 and 9). In the former case the two bars produced by the division of one simply spread apart and at the same time broaden out; in the latter case the small alternate new bars merely increase in size until they become nearly as large as the original bars, although it is usually easy to distinguish the latter by their greater length. In one unusually large male I observed fourteen well developed bars nearly all of which had begun to split at the ends as though preparing to double the number of bars once more. As a rule the most anterior bars are the first to show signs of splitting, the tendency proceeding antero-posteriorly. It seems to be a very general rule that meristic changes of this sort proceed in this direction. The color pattern of the females is at first similar to that of the young male, but during the second season, probably, a marked change begins to take place. The eight or ten bars that exist at that time show decided irregularities in outline, each bar, beginning with the most anterior, sending out at two places forward and backward processes, which, on examination, prove to be arranged in two longitudinal lines, the upper one on a line with the eye and the lower one on a line with the angle of the operculum (Text Plate V., Fig. 2). The processes especially those of the upper bar, continue to elongate anteriorly and posteriorly until those of adjacent bars fuse together into a continuous longitudinal stripe, the remaining portion of the bars becoming attenuated and



TEXT PLATE V.

Showing stages in the development of the adult male and female color pattern in *Fundulus majalis*. These are camera drawings made from formalin preserved specimens except in two cases 1 and 9 which are taken from free-hand drawings of living fish. 1½ natural size.

1. A young fish of undetermined sex, showing the type of pattern seen in all young fish.

2 to 5. Stages in the development of the female color pattern. The gradual change from cross bars to stripes is clearly brought out.

6 to 9. Stages in the development of the most specialized male color pattern seen in 9. Not many males reach this stage.

gradually fading out (Text Plate V., Fig. 3). The lower line of processes behaves in a slightly different manner. Instead of spreading out to form a continuous straight band it forms a series of short, nearly longitudinal bars (Text Plate V., Fig. 4). Later on these short bars fuse end to end in various fashions to form from two to five longer bands or stripes one of these extending nearly two thirds of the length of the body and the others, some above and some below the lower long stripe, vary greatly in length in different individuals (Text Plate V., Fig. 5). This process of conversion of bars into stripes takes place, as in the case of the doubling of bars in the male, in an anteroposterior direction. In half grown females all stages of the process may readily be found. Such specimens show from two to five of the posterior bars intact while the stripes at the anterior end are well marked. I have never yet seen an adult female in which all of the bars had disappeared, the most posterior one always persisting. The appearance of adult females is strikingly characteristic and must serve as a very efficient recognition mark for the males. The sexual difference is accentuated by the fact that the fins of the female are nearly or quite devoid of any dark pigment, only a small amount of a light yellowish pigment being present and that chiefly on the caudal fin.

The fins of the male bear the same characteristics as do those of *F. heteroclitus*, but are even more pronounced in their sexual dimorphism. Especially is this the case in the anal fin which is prolonged backward more markedly than in the previously described species (see Plate XXVII., Fig. 4). The anal fin of the female is swollen and inflamed in egg-laden specimens freshly brought in. The flow of milt is free and copious only in males possessing the highest coloration. The contact organs are even better developed than in *F. heteroclitus* and their distribution is very similar.

Beyond these indications I have no direct evidence bearing on the spawning behavior of *Fundulus majalis*. Although taken in large numbers when they were apparently at the height of their sexual activity, as indicated by the abundance of ripe eggs and sperm, they showed no tendency to spawn in captivity. The fish are much wilder than *F. heteroclitus* and seem to feel captivity much more keenly. All of their normal activities seem to be, for a time at least, inhibited by confinement in restricted quarters, as is indicated by the fact that they will not feed until they are nearly starving, so it is hardly to be expected that they would spawn under these conditions. I think there can be no reasonable doubt, however, that their spawning behavior closely resembles that of the species described, especially since the observed behavior of a representative of another genus, viz., *Cyprinodon variegatus*, so closely resembles that of the latter. It is highly probable, judging by the very much elongated anal fin of the male *F. majalis* that this fin plays a more important part in clasping the body of the female than does the analogous fin of *F. heteroclitus*, but the difference, like that in the other details of spawning behavior, is probably one of degree rather than one of kind.

Fundulus Diaphanus.

The following extracts, referring to sexual dimorphism, are taken from Jordan and Evermann's systematic account:

> "Fins not large; dorsal and anal rather low; ventral scarcely reaching vent in females; somewhat longer in the males. General color olivaceous; sides silvery. Male with about 20 silvery vertical bars, narrower than the dark interspaces; female with 15 to 20 transverse bars, shorter than the silvery bands of the male, the interspaces pale; back sometimes spotted; young always with black bars; fins nearly plain."

In the above description the dark coloration is arbitrarily spoken of as the background on which are superimposed silvery bands, in the case of the male. In the case of the female, on the other hand, the silvery is referred to as background for the somewhat narrower dark bars. A casual examination of the figures representing the two sexes (Plate XXVIII., 1 and 2) will show, I believe, that the dark bars are analagous in both male and female, those of the former simply being considerably broader and a little darker than those of the latter. This greater distinctness of the cross banded pattern in the males is just what we should expect to find if we compare this with the other species examined. The dimorphism in color pattern, however, has not become so pronounced in this case as in the others, and probably represents a more primitive condition.

The dimorphism in the case of the dorsal and anal fins, although not nearly so marked as in the other species, is still quite evident, especially in the case of the anal, which is considerably longer in the male. That of the female, as in the other species, is stiff and swollen, but only in the basal two thirds. The dorsal of the male is frequently colorless or nearly so, like that of the female, but is often decidedly mottled with dark pigment after the fashion seen in the male of *F. heteroclitus*, but much more lightly. The chief mottling is found on the posterior rays, a sort of prophecy of the very marked spots on this fin in other species. The contact organs are similar in form and distribution to those of *F. heteroclitus*.

In general it might be said that this species shows the beginnings of sexual dimorphism in practically all of the points that become so marked in other species, and probably represents a primitive condition.

I am sorry to be unable to present any facts regarding spawning behavior, but must plead as an excuse that this species, being a fresh or brackish water form found in a large pond on Martha's Vineyard, was inaccessible for observation in its natural haunts, and could be transported to aquaria only with the greatest difficulty. Moreover, when once they are transported and are safely housed they die off very rapidly and the diligent collector receives a poor reward for his labor. The species is not at all resistent to adverse conditions as is *F. heteroclitus* or even *F. majalis*.

The presence of a sexual dimorphism, the same in kind although different in degree, from that in other species of the same genus, lends probability to the belief that the spawing behavior is similiar to that described above.

CYPRINODON VARIEGATUS Lacépède.

(Sheephead Minnow; Purcy Minnow; Short Minnow.)

Spawning Behavior.

The opportunity was not afforded me of observing the behavior of this species in its native environment, but I was very fortunate in being able to get fairly complete records of its spawning habits by the use of aquaria.

The following observations were made on July 20:

A medium-sized aquarium was fitted up with stones *Ulva*, *Fucus*, etc., to approximate natural conditions, and five male and nine females of the species *Cyprinodon variegatus* were introduced. At about eight o'clock in the morning, when all was quiet in the room I had a fine opportunity of observing the spawning behavior of these fish. Of the five males three were in spawning plumage. one of them being noticeably more brilliant than the other two. When I first observed these fish this brightest male had acquired complete control of the situation. He was so extremely active and pugnacious that he succeeded in driving not only the other males but the females into the friendly shelter of the masses of coarse brown seaweed. Whenever any of the other fish so much as ventured to poke its head out of the shelter the ruling male would dash up and scare it into its retreat again. One of the brighter males, less subdued than the rest, ventured out more frequently and farther than the others. Instead of meekly retiring before the vigorous onslaught of the enemy, he offered considerable resistance. On several occasions the encounters between the two males developed into combats at close quarters, in which heads and jaws were the weapons. They would begin by butting heads fiercely and would occasionally grasp jaws and shake one another powerfully from side to side as though each were endeavoring to tear out his opponent's jaw. These struggles were usually short lived, one of the belligerents, invariably the one that had been in hiding, seemed to tire. They would then separate, as though by mutual consent, and the defeated male would ingloriously retreat to shelter, usually slowly as though exhausted. I have been unable to notice that either of the combatants in these frays receive any injury.

It is, I believe, more of a test of vigor and endurance than any attempt to inflict bodily injury. On the supposition, then, that sexual vigor and general bodily vigor run parallel and that the index of both is the brilliancy of coloration, we can readily understand why it is that the most brilliantly colored male is invariably the victor in these struggles for supremacy. It probably is the case too that a higher courage accompanies a higher bodily and sexual tone and makes a male at his climax practically invincible. It seemed to me, as I watched the activities of this male, that his extreme impetuosity was decidedly a detriment to him, for he defeated his own ends by driving away all females that ventured to seek the open. The ruling male always approached these females, which were ready to spawn, judging by their distended abdomens, with so much fierceness and speed that they were forced to retreat. Occasionally this male succeeded in cornering a female in one of the angles of the aquarium either at the bottom or at the sides, and spawned with her. The method of spawning is not unlike that observed in *Fundulus heteroclitus.* The male holds the female just forward of her caudal fin, using chiefly his very large, strong dorsal fin for this purpose. He lies slightly on the back of the female, but mainly side by side with her. The anal and ventral fins are used to hold the female up against the clasping dorsal, but these fins do not, so far as I was able to observe, clasp so firmly as in *Fundulus.* The approximation of sexual openings is not nearly so close as in the last-named species and hence there is probably less surety of successful fecundation. The eggs and sperm are, however extruded in such close proximity that the chances of failure are comparatively slight. While clasped by the male the female vibrated the body as described for *Fundulus* but more rapidly. In fact the whole spawning act is of such short duration that it is extremely difficult to see exactly what takes place. One has to make repeated observations, watching each detail of the process and even then some details elude ones most careful scrutiny.

July 22. — Two days later the ruling male still continued to rule although challenged frequently by the other male, now almost equally brilliant. The encounters between these two belligerents was very interesting, reminding one of nothing so much as an encounter between two game cocks. They approach threateningly, every fin erected and body quivering for the fray. Then they dash at one another, the ruling male being slightly more aggressive. After an encounter, much more evenly waged than on the former occasion, the rival male gives up temporarily and retires to his corner. He is still full of fight, however, for in a few moments he begins to make threatening demonstrations in his corner, turning sudden summersaults and making quick, active darts out and back. This behavior never fails to bring his opponent to the fray again. This sort of thing usually lasts until one of the combatants — always the lesser male — grows weary and retreats to a convenient hiding place, there to recuperate for another series of encounters. Several other fairly brilliant males could be made out, hiding in the same fashion. The females are still too frightened to emerge from shelter. The fierce aspect of these fighting males is remarkable in creatures so small. When swimming ordinarily the dorsal fin is not used, being kept folded flat on the back. But whenever fighting or sexually excited, this fin comes into action, expanding like a bat-wing sail.

The reckless courage of the ruling male surprised me. I put into the aquarium a male *Fundulus hereroclitus* of large size. Without a moment's hesitation the little warrior dashed up to the far bulkier intruder and caused the latter to beat an ignominious retreat. Several larger fish of other species met a similar reception and fate.

July 23.— On the following day the ruling male had been deposed and there was an extremely vigorous struggle for supremacy among the other males. Only one of these failed to enter the lists. This one had as yet not reached the sexual climax and was, consequently *hors du combat.* As one might have anticipated, the male that had ousted the original ruling male was the victor, the others finally acknowledging defeat by retiring to shelter. The same program was then repeated that has been described for the previous days.

The deposed male now showed signs of waning vigor in the paling of his " plumage," which was becoming rather dull at the posterior end of his body. This fading out of brilliancy proceeds in a postero-anterior direction, the top of the head being the last part of the body to lose its bright coloration.

July 29.— Both males and females of *Cyprinodon* are seen to have passed the sexual period. The females are no longer distended and no longer show any fear of the males, the two sexes mingling quite amicably. The males have all lost their spawning coloration except for traces of iridescent green about the head. The dorsal fin of the males, having lost most of its pigment, shows in one or two cases quite a noticeable dark spot on the posterior rays. This spot is, however, not nearly so distinct as that in the females. In color the males are now pale green with dusky markings that stand out somewhat more clearly now that the iridescence and general dark body coloration has nearly disappeared. The males are still greener than the females but the difference in the intensity of the coloration is far from marked.

When the fish were fed it was noticeable that the males always erected the dorsal fin when they made a dash for a fragment of food. They seem to raise this fin whenever excited in any way.

Sexual Dimorphism.

The following extracts referring to sexual dimorphism are taken from Jordan and Evermann's systematic account:

> ‘‘Body very short and robust, in adults high and much compressed, the females abruptly constricted at the base of the caudal peduncle. . . . Dorsal fin moderate, in females as high as the length of its base, in males much higher; origin of dorsal midway between base of caudal and end of snout; base of fin $1\frac{1}{3}$ to $1\frac{2}{3}$ in length of head; longest ray (in male 2 inches long) reaching half way from base of fin to base of caudal, the anterior rays equaling length of head and extending beyond tips of posterior rays when the fin is depressed; in females the longest ray about $1\frac{1}{2}$ in head; origin of anal under eighth or ninth ray of dorsal, the fin very small and much higher than long; length of base about equaling snout; longest ray half length of head (less in females). No external oviduct. Caudal truncate or slightly emarginate, $1\frac{1}{4}$ in head; ventrals, in adult males, reaching in front of anals, $2\frac{1}{3}$ in head; in females reaching vent; . . . Scales large, tuberculate in males, arranged in regular series; . . . Color: Male olivaceous; from dorsal forward above pectoral to head deep, lustrous steel blue, the color very intense and conspicuous in life; rest of upper parts with rather greenish luster, becoming dull slaty blue, and on cheeks, opercles, sides anteriorly and belly deep salmon color; lower lip and preopercle violet; dorsal blackish, the anterior margin of fin orange; caudal dusky olive with jet-black bar at tip, and a narrow black cross-streak at base; anal dusky at base, bordered entirely around with bright orange; ventrals dusky, bordered with orange; pectorals dusky orange, darker below. Smaller specimens show some orange shading on the sides, and sometimes also traces of the cross-bands of the female. Female very light olive; lower half of the sides with about 14 alternately wide and narrow vertical, dark bars, those anteriorly narrower and closer together; usually 7 or 8 dark cross-bars on the back, alternating with the wide bars below; these bars are of various degrees of distinctness, sometimes almost obsolete; a dusky area below eye; young with broad greenish cross shades wider than the interspaces; belly pale or yellowish; lower jaw largely blue; cheeks brassy; dorsal dusky, with an intense black, faintly ocellated spot near tip of last rays; caudal faintly reddish, with a black bar toward base; other fins pale orange, with some dark points. Length: Male 3 inches; female

2 inches. Cape Cod to the Rio Grande, in brackish waters, entering streams, very abundant southward, the males more highly colored southward, but the southern form (called *gibbosus*) not otherwise different.''

These sexual differences, especially those of general bodily form, comparative size and shape of fins, color pattern, etc., are well brought out in the illustrations (Text Plate II., Figs. 3 and 4). Characters involving color, iridescence and the like cannot be represented in monocrome, so it will be necessary to fall back upon verbal description. For this purpose I cannot do better than to refer to Jordan and Evermann's full account for details not brought out in the illustrations. This account was evidently written with reference to the fish when in "spawning plumage," no reference being made to the fact that, in the males especially, the color and iridescence are merely temporary adornments, characteristic of the breeding season alone. Before and after the breeding season the males are about the same color as the females — perhaps a trifle greener.

The points of sexual dimorphism to be especially born in mind are the following:

1. The male is usually somewhat larger than the female — the opposite being the case in the species of *Fundulus* described.

2. The male, in "spawning plumage," is very much more brilliantly colored than the female.

3. The body of the male is decidedly deeper but more compressed than that of the female, differing from *Fundulus* in this respect.

4. The dorsal, anal and ventral fins are larger in the male than the female, even more markedly than in *Fundulus*.

5. The cross-barred color pattern is retained more nearly intact in the female than in the male, the opposite condition holding for *Fundulus*.

6. The dark, ocellated spot, that, in *Fundulus* characterizes the posterior rays of the dorsal fin of the male, is present here only in the female, although the same area in the male is usually more heavily pigmented than the rest of the fin in the male.

7. The contact organs are similar in form and distribution to those of *Fundulus*.

8. As in *Fundulus*, all of the fins of the male are more deeply and more brilliantly colored than in the female.

9. The generally aggressive and warlike aspect of the male is in striking contrast to the comparatively mild and timid aspect of the female. This contrast is more striking in *Cyprinodon* than in the species of *Fundulus* examined.

10. This exceedingly fierce aspect of the male is largely due to the very marked height of the dorsal fin and his method of carrying it spread to the utmost.

Summary and Conclusions.

Classification of Secondary Sexual Characters.

I. Of the permanent differences the following is a list:

1. Relative size of the sexes.
2. General body form.
3. Relative sizes of the various fins.
4. Shape of the various fins.
5. Color pattern (*a*) on the body, (*b*) on the fins.
6. Quality and intensity of coloration, whether it consists of pigmentation or of iridescence.
7. Relative abundance of the sexes.

II. Of the temporary differences that appear only during the spawning season the following is a list:

1. The distended abdomen of the female.
2. The swollen and inflamed anal fin in the females of *Fundulus*
3. The marked intensification of coloration and iridescence in the males, both on the body and on certain fins.
4. The paling of color in the females.
5. Contact organs in spawning males.
6. The increased activity of the males, accompanied by an increase in courage.
7. The coyness of the females, especially in *Cyprinodon.*

All of these secondary sexual characters can, I believe, be shown to be either direct adaptations to the sexual life of the fish or necessary accompaniments of the high physiological tone that accompanies the sexual climax.

Of the permanent characters the most obviously adaptive are those that have to do with the differences in size of certain fins in the two sexes. Without reasonable doubt the enlarged dorsal, anal and ventrals are adaptations to facilitate clasping. The origin of this adaptation and the way in which it is thought to have given rise to the habit of intromission will be discussed later.

The differences in shape of certain fins may, with equal certainty, be said to be adaptive. The backwardly directed prolongations seen on the anal fins of all the species of *Fundulus* examined are evidently to give the male a greater reach in his effort to clasp firmly the body of the female. The shortness and softness of the dorsal fin of the female is of advantage in that it is thus less in the way.

Differences in color pattern can only be explained as sex recognition marks. It can readily be seen, in the light of the observations on the spawning behavior of these fish, that it must be possible for the males to recognize the females at once and at a distance. Some distinctive character that would appeal to the visual sense is required. In the course of evolution those females that could readily be recognized as females would be the ones most frequently mated with and they would be the most likely to transmit this variation to their descendants. There seem to be at least two means of acquiring a distinctive appearance that would serve to mark off the females from the males. One means is to lose the common racial marking more or less completely, and thus to acquire a sort of secondary solid coloration. This method can be seen in two stages of development in two species of *Fundulus*, viz., *F. diaphanus* and *heteroclitus*. The former presents a condition in which the bars of pigment of the female are simply narrowed markedly, while the latter shows their reduction to the merest suggestion of a cross-banded pattern. The second means of acquiring a distinctive female marking is exemplified by *F. majalis*, in which the characteristic banded pattern of the species, which is possessed by the young of both species, is, during ontogeny, gradually converted into a longitudinal striping of a most pronounced order. The stages in the process of change from a transverse to a longitudinal pattern have been described in another place.

Attention has been called to the sequence of changes from the young to the adult color pattern in both male and female of *Fundulus majalis*. In the first place there seems to be a very deep-seated law of antero-posterior development. In the second place there is in the female a transition from a primitive cross-banded pattern to one characterized by longitudinal stripes. This is the opposite order of change from that given by Eimer and shows that his laws of orthogenetic variation have only a limited application. We have here a clear case of orthogenetic variation during ontogeny, a phenomenon that Gadow tried unsuccessfully to show in the case of scutes of *Chelonia*.

The total male plumage cannot be considered as primitive, the spawning plumage proper being secondary, an especial male acquisition due to his superabundant vitality. The cross-banded pattern is probably primitive and was possessed by both sexes. Even the distinct spot on the dorsal fin in the males of various species of *Fundulus* was probably a character common to both sexes, for in the allied species *Cyprinodon* we find this marking more pronounced in the female, but often present in rather vague form in the males.

Whether differences in the relative abundance of the sexes can be explained as adaptations is open to discussion. The fact that, in all the species studied, the males are relatively rare may be explained by the law of economy, for comparatively few males are quite capable of fecundating the eggs of many females, hence any more males would be superfluous. The experiment of putting too large a proportion of males in aquaria with females shows the disadvantage of a superfluity of males, for they spend most of their time fighting instead of devoting their attention to spawning.

The more slender body form of the males in *Fundulus* and the deeper but more compressed bodies of the males of *Cyprinodon* are probably both adaptions to the more active and combative disposition of that sex.

Of the temporary characters that accompany the sexual climax in both sexes, the heightened color of the males is most readily explained on purely physiological grounds. It is well known that heightened vigor, whether reproductive or somatic, is accompanied by a more active metabolism, and it is equally well known that pigmentation is a sort of index of the rate of metabolism in an organism. A heightened sexual vigor is then necessarily accompanied by an increase in pigmentation. So much for that phase of color intensification that is simply dependent on an increase of pigment ; the blue gleam that appears only during active spawning must be the direct result of sexual excitement, for it fades almost immediately when the males are removed from the sexual environment. I am inclined to look upon this gleam as a sort of flush such as might suffuse the human body under excitement.

Contact organs may be considered as excrescences produced by the excess vitality of the male and specialized for an especial function. As suggested in the introduction of this paper, it is believed that both structure and function appeared in response to a heightened metabolism. After their appearance the structures were modified by use or function.

It is interesting to endeavor to trace the origin and development of the habit of intromission that seems to prevail among about half of the Pœciliidæ. This habit is invariably associated with an increase in the length of the anterior rays of the anal fin and the modification of these rays in various ways into an organ of intromission. The first step in the process was doubtless a mere elongation of the whole anal fin, as seen in *Fundulus diaphanus*. The next step was probably a more rapid growth of the anterior rays of the fin, such as we see in *Fundulus heteroclitus* and to a slightly more marked extent in *Fundulus majalis*. The function of this elongation in these species of *Fundulus* is partially to give the male a greater reach and partly to fan the sperm toward the extruded eggs. It is only a few steps farther in the same direction for the anal fin to assist the sperm to enter the oviduct of the female when internal fertilization becomes necessary.

PLATE XXVII.

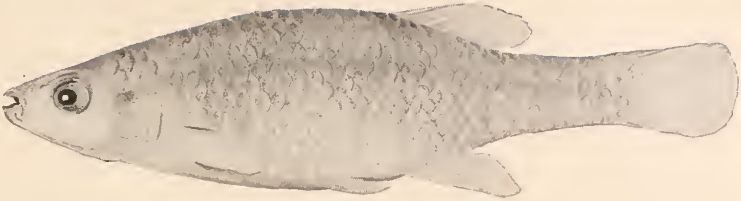
FIG. 1. *Fundulus heteroclitus*, female.

FIG. 2. *Fundulus heteroclitus*, male.

FIG. 3. *Fundulus majalis*, female.

FIG. 4. *Fundulus majalis*, male.

These figures and those in Plate XXVIII. are reproduced from wash drawings and represent the actual sizes of the average adults.



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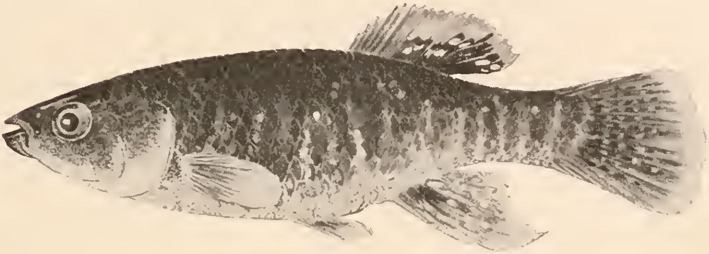

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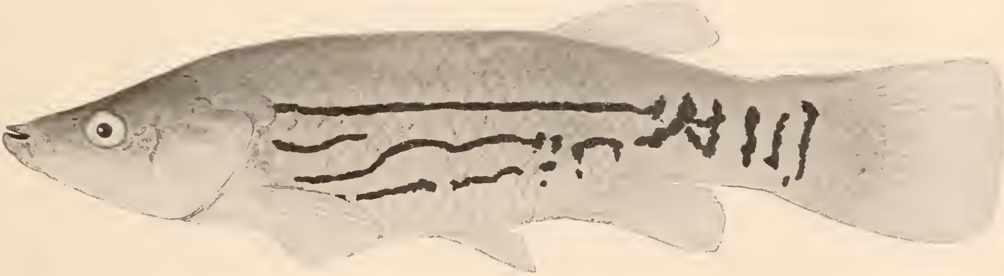

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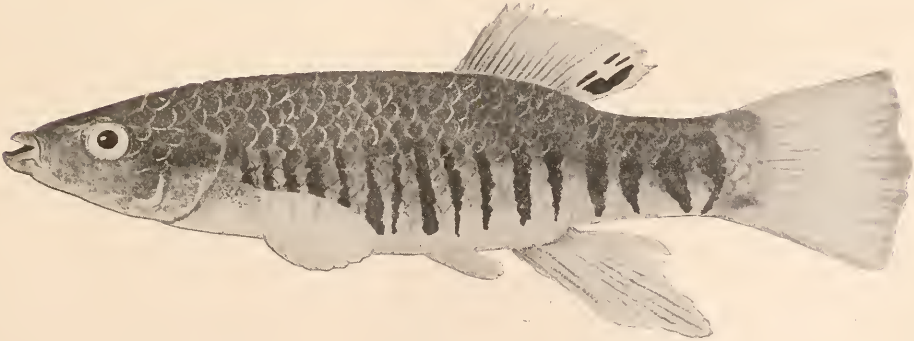

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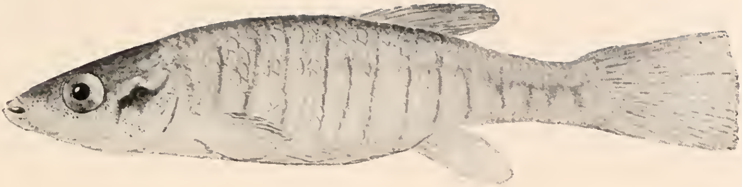
PLATE XXVIII.

FIG. 1. *Fundulus diaphanus*, female.
FIG. 2. *Fundulus diaphanus*, male.
FIG. 3. *Cyprinodon variegatus*, female.
FIG. 4. *Cyprinodon variegatus*, male.



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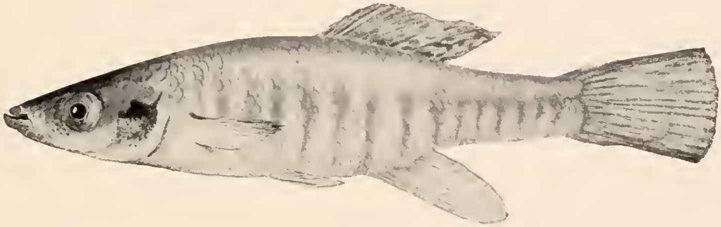

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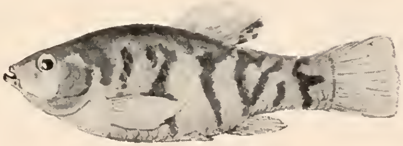

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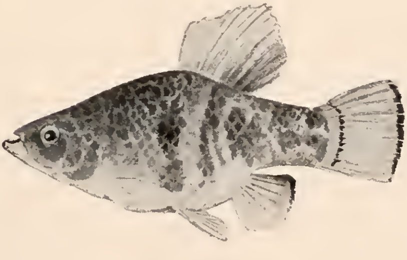

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Vol. XII. May, 1907. No. 6

BIOLOGICAL BULLETIN

THE SYSTEMATIC AFFINITIES OF THE DIPTEROUS FAMILY PHORIDÆ.

CHARLES T. BRUES.

In a paper on the nomenclature of the dipterous wing published in the current number of *Psyche*, Professor Williston ('06) has again raised the much vexed question of the relationship of the Phoridæ among the families of Diptera.

During the past few years I have devoted considerable time to a study of this small and truly remarkable group of flies, but have been unable so far to reach any wholly satisfactory conclusion as to their proper systematic position. Attempts in a number of directions have each revealed some important if not insuperable obstacle, and I had let the matter rest, compelled to accept, albeit rather unwillingly, the final decision expressed by the late Baron Osten Sacken in the following words: "A real *affinity* with *Phora* does not exist anywhere."

In the nature of things such a negative opinion on a question of phylogeny can only be tentative, and it is with pleasure that I find the discussion reopened by so eminent a dipterist as Professor Williston.

Several entomologists who have given special attention to the group have attempted to reconcile its notable peculiarities of structure with types to be found elsewhere within the order of Diptera, and one has even essayed to connect them with the Aphaniptera which are almost unanimously regarded as forming a separate order.

The antennæ and the wings are the organs which offer the difficulties and I shall describe them briefly. The antenna consists apparently of a single large globular or pear-shaped joint which bears a dorsal or terminal arista composed of three joints. When examined more closely, however, and more especially in longitudinal section, it is seen that this large segment is complex (see Fig. 1) and consists of a smaller joint almost entirely concealed within a larger outer one. These two are attached to the head by a third small and partially concealed segment. Such an arrangement is constant throughout the Phoridæ and is, so far as I know, absolutely unique among Diptera.

The nomenclature and homology of these joints has been interpreted in several ways. Wandolleck ('98) has considered the antenna to be six-jointed without attempting to homologize the segments with those of the antennæ of other Diptera. The majority of recent dipterists seem to have accepted a nomenclature which would regard the three large basal joints as homologous with the three segments of the typical brachycerous antenna, the apical one supplied with an arista of the usual kind. The third interpretation suggested in the paper before referred to by Williston ('06) is that the basal portion consists of two joints only, and that the arista so-called really represents the whole flagellum of the nemocera. Schiner also refers to the antenna as apparently two-jointed ('64, p. 335), but on page xv he says in more detail: "Fühler nahe am Mundrande eingefügt, scheinbar zwei-gliedrig, das dritte Glied rund (bei *Conicera* kegelförmig), mit nackter oder doch nur pubescenter Rücken- oder Apicalborste." It is thus evident that he takes them to be actually three-jointed, and in this view I fully concur. From the examination of large numbers of mounted specimens representing most of the described species, as well as several in microscopical section, and a number of published figures, I am fully convinced that there are always three distinct joints, the large apical one of which bears an arista.[1]

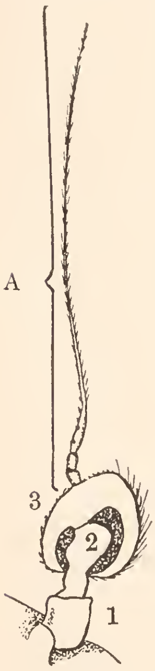

FIG. 1. Phorid Antenna. 1, 2, 3, antennal joints; *A*, arista.

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[1] Whether we conceive the arista to be a modified portion of the nemocerous flagellum would in this connection be a problem apart from the relationship of the various brachycerous families among themselves.

In all species that I have examined the arista is three jointed, the two basal joints short and the other long. It thus offers no noticeable difference from the same appendage of many typical Brachycera.

Williston suggests that the Orphnephilidæ have antennæ comparable to those of the Phoridæ except for the number of aristal joints which is seven. In this case however, to judge from Schiner's description[1] the second joint is composite like the third joint of the Tabanidæ, Stratiomyidæ and allied forms. Whether the Orphnephilid antenna is in reality three-jointed I am unable to say, but the annulation of the second apical joint finds no counterpart among the Phoridæ.

Williston believes that the palpi are two-jointed, and bases a part of his argument for nemoceran affinities on this supposition. From my own observation I do not believe however, that such is the case, nor can I find any reference to this effect in the literature and published figures at hand,[2] with the exception of a single diagram by Dahl ('99, p. 75, Fig. 5) where he figures the palpi of the East Indian *Phora* (*Dorniphora*) *dorhni* with two joints. I think the accuracy of this one case can be questioned as I have identified almost certainly his species among a lot of Phoridæ from New Guinea and find the palpi single jointed as usual. Becker ('01) gives careful figures of the head with palpi in a number of genera[3] but in each case there is no indication of more than a single joint. I have studied with great care the mouthparts of several of the subapterous myrmecophilous genera, but have found no trace of a second joint. Fig. 2 is reproduced from a drawing of one of these species in which there is no trace of any articulation. The palpi of the Phoridæ are always large, and are in some instances immensely swollen (*e. g.*, *Phora palposa* Zett. and *Aphiochæta magnipalpis* Aldrich ♂) but in other respects they differ from those of the more specialized Brachycera like the Muscidea only in their more strongly developed bristles.

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[1] In "Fauna Austriaca," II., 643, he says of the Orphnephilidæ: Fühler nahe am Mundrande stehend, kurz und so sich darstellend, als ob sie aus einem runden ersten, einem ovalen zweiten und einer Endborste bestünden; bei mikroscopischer Untersuchung zeigt sich, dass das zweite ovale Glied aus drei und die Endborste aus sieben walzenförmigen Gliedern besteht, deren letztes am Ende borstig ist.

[2] After this paper had gone to press, Dr. Williston called my attention to another published paper by Wesché (*Journ. R. Micr. Soc.*, 1904) in which Phorid palpi are figured as two-jointed.

[3] *Phora*, *Hypocera*, *Aphiochæta*, *Trineura* and *Metopina*.

It is in the venation of the wings that the Phoridæ depart most strikingly from the other Diptera. This varies among the few known genera only in trivial details, so that the general type for the entire family is practically uniform. Fig. 3 shows the wing of a typical species. The veins can at once be divided into two groups, the several heavy ones which lie close together at the basal half of the wing anteriorly, and the four delicate ones which pursue an oblique course across the posterior part of the wing. This arrangement is so peculiar, that attempts to homologize the veins with those of other Diptera have proved very unsatisfactory. Several families present a somewhat similar appearance and in each case comparisons have been drawn. Williston, in the paper referred to above ('06) mentions the close similarity in venation to the Bibionid genus *Apistes*, and Becker ('01) earlier noted the resemblance to *Scatopse* another member of the Bibionidæ and to *Simulium* belonging to the Simuliidæ. It is true that there is a close superficial resemblance but no attempt has been made to homologize the veins. It is evident that *Apistes*, *Scatopse* and *Simulium* are all considerably specialized and we can indeed trace through the Bibionidæ the loss of the posterior series of veins which are quite strong in *Bibio*, weaker in *Dilophus* and still more so in *Scatopse* and *Apistes*, with a coincident strengthening of the anterior veins and their withdrawal basally. That a resemblance between this, the result of specialization within a family of closely similar forms and another otherwise widely different family

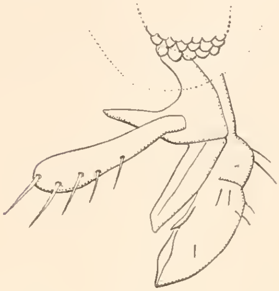

FIG. 2. *Commoptera solenopsidis* Brues, mouthparts of ♀.

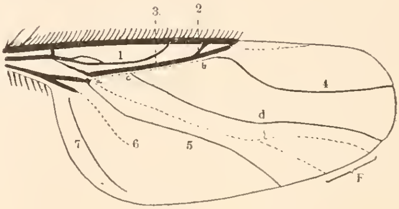

FIG. 3. Diagrammatic Phorid Wing. (After Becker.)

can have phylogenetic import, I am inclined to doubt. I think it can not be questioned that a complete venation is the primitive condition for the Bibionidæ, so that we cannot assume a common origin for the Bibionidæ and Phoridæ on evidence from neuration, but necessarily a *Scatopse*-like or *Apistes*-like ancestor for the Phoridæ. This is manifestly improbable as it involves after the fixation of a degenerating wing venation, vast strides in the evolution of other organs (antennæ, palpi) to give rise to Phoridæ while the parent stock remains unchanged.

Such a loss of posterior veins has occurred independently in many groups; the Stratiomyidæ and Hippoboscidæ may be mentioned among the Diptera and the Chalcididæ, Proctotrypidæ, Bethylidæ and Cynipidæ among Hymenoptera. The adaptation seems to be due to mechanical adjustment and of course not to common ancestry.

Palæontology offers but little on the history of the Bibionidæ and practically nothing on that of Phoridæ. The occurrence of *Phora* has been recognized by Berendt ('45) and Loew ('50) in Prussian amber. The bibionid genera *Protomyia*, *Bibio*, *Plecia*, and *Bibiopsis* have been found to occur together in considerable abundance in the Mayencian formation at Radoboj in Croatia (Heer, '47), while *Scatopse* is known first from the Ligurian, occurring in Prussian amber with *Phora* and also at Aix, France (Serres, '29).

In his monograph of the European Phoridæ, Becker has advanced the idea that the Phoridæ are derived from nemocerous forms allied to the Mycetophilidæ, basing his opinion principally upon a careful comparison of the wing venation of the two families made by Girschner. Girschner's view is given as follows: "Ich halte das Phora Geäder für ein modificiertes Mycetophilidengeäder, wie ich auch die Mycetophiliden für die nächsten Verwandten und Stammesangehörigen der Phoriden halte. Ich deute das Geäder in der oben skizzierten Weise (reproduced here as Fig. 3). Von *a* bis *b* ist die Discoidalader mit der Cubitalader verschmoltzen, wie dies schon angedeutet wird bei Macrocera. Bei einigen Phora-Formen kann man ziemlich deutlich — doch nicht so auffallend wie in der Zeichnung punctiert angegeben — die unter die Cubitalader hinstreichende Discoidalader wahrnehmen.

Die kleine Querader fehlt, weil die in der Strecke *ac* enthalten ist."

This comparison is very striking and were it not for the fundamental differences in metamorphosis and in other organs, might be conclusive. It is further strengthened by the fact adduced by Becker that certain of the macrochætæ covering the body are in both families provided with chitinous bristle-like projections.

On the other hand, Schiner has with almost equal facility reduced the Phorid wing to a more generalized type of brachycerous venation according to the following scheme (see Fig. 4).

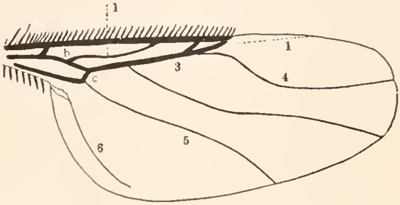

FIG. 4. Wing of *Phora* sp., as interpreted by Schiner.

The main differences between this and the arrangement by Girschner lie in the interpretation of the fourth vein involving the presence of the cross-vein and the presence of the anal vein. Schiner's designation of the indistinct thickening along the costa beyond the tip of the heavy veins as a "Fortsetzung der ersten Längsader" I cannot exactly understand, but it involves no important point.

Of the two views, that of Girschner accords more closely with the actual wing venation, and will I think be partly accepted with the exception of several points mentioned on a later page, by all who will study carefully a series of wings. That this similarity between the Mycetophilid and Phorid wing involves genetic relationship does not necessarily follow, however. As suggested on a previous page, specialization of wings often follows more or less parallel paths in diverse groups, and this seems to be especially true in those where a loss in complexity of structure is brought about by parasitism, secretive habits, or minute size. Turning to the Dipterous family Hippoboscidæ, widely removed from those previously discussed, we can see a strikingly similar condition to the one assumed by Girschner for the Phoridæ. Fig. 5 shows the wing of a species of *Olfersia*, a genus of Hippoboscidæ. The heavy veins are confined to the anterior basal region, while several oblique light veins traverse the large posterior expanse. Moreover, the individual veins retain almost the same relative position in these representatives of the three families. In *Olfersia* the first, second and third longitudinal veins join the heavy costal vein, which extends for only a part of the wing length, in nearly parallel directions. The fourth is not fused with the third basally, but lies close to it so that the anterior cross-vein is extremely short. Such precisely similar developments are remarkable, and must, I think, be due to some similar tendency to degenerate in certain definite directions.[1]

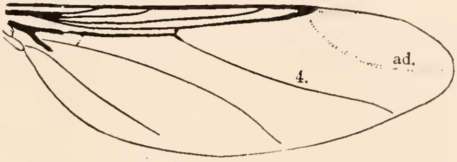

FIG. 5. *Olfersia* sp., wing. *ad*, adventitious vein ; *4*, fourth vein.

Be this as it may, I think the evidence is sufficient to show that direct descent from Mycetophilid-like forms is not a logical necessity in tracing a genealogy which will account for the similar wing venation of the Phoridæ.

Several other attempts to discover relationships that have been less elaborately worked out are enumerated in the papers by Osten Sacken ('02) and Coquillett ('01). There is no agreement as to whether they belong properly to the Orthorrapha or Cyclorrapha. Osten Sacken (*l. c.*) refers them to his orthorraphous superfamily Energopoda which contains the Asilidæ, Empididæ, Lonchopteridæ and Phoridæ, while Coquillett has proposed the orthorraphous superfamily Phoroidea to contain the Lonchopteridæ and Phoridæ. Mik thought he saw Borborid affinities, as he says ('98, p. 205): "Aenliche Mundtheilen . . . findet man bei den Borboriden, mit welchen die Phoriden wahrscheinlich nahe verwandt sind."

The concensus of recent opinion associates the Lonchopteridæ and Phoridæ together with apparent good reason. The head, form of front with its macrochætæ, proboscis, palpi, antennæ with their arista, bristly thorax and legs, large, freely articulated anterior coxæ, and the general habitus and actions when alive are very similar in both families. On the other hand the wing venation is different, but taken in connection with the facts already presented showing similar degeneration in so many families, this is not so great an objection as it would be otherwise.

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[1] Such an idea savors of orthogenetic principles, although in an unusual sense, offering an interesting field for speculation, and for comparative studies as well.

Aside from Mik, dipterists do not appear to have detected many affinities with muscid forms. I am nevertheless inclined to agree with him that the Phoridæ show a relationship with the Borboridæ. This may quite probably also have been Schiner's idea when he placed the Phoridæ between the Bibionidæ and Borboridæ, rather than to show relationship to the former family as Williston suggests ('06). The various points of resemblance are: first, their similar appearance and actions when alive, they are the only family whose motions when running or flying could be confused with those of the Phoridæ; the modification of the incrassated hind metatarsi, the same segment being modified by rows of scaly bristles in every member of the Phoridæ, even the wingless forms and Termitoxenia; the tendency shown in the Borboridæ to lose certain of the apical and posterior parts of the veins in the wing and the tendency also to develop wingless forms like the Phoridæ. However, the legs and head of the Borboridæ are not bristly to any extent. Other differences are mainly the ones that appear equally in comparison with any family.

It is therefore in my opinion probable that the Lonchopteridæ and Phoridæ may together find a more suitable place in the Cyclorrapha rather than at the end of the Orthorrapha where placed by Coquillett. This is what Williston has done with them in the second edition of his manual ('96) where they are placed between the Platypezidæ and the Muscidea. At least there seem fewer objections to this course than to any other.

Regarding the nomenclature of the wing veins of the Phoridæ there has been considerable difference of opinion as mentioned on a previous page, and I would like to correct what I think is an error in the identification of the fourth and fifth veins. The first oblique light vein has been considered by various writers (Schiner, '62, Comstock, '94, Becker, '01, Brues, '04) as the fourth longitudinal vein, but I now believe that the second light vein represents the fourth and that what has been called the fourth is an adventitious vein. The process of coalescence in the Hippoboscidæ has suggested this.[1] Here (see Fig. 6) the first light vein arises at the cross-vein which is near the middle of the third vein. This vein is undoubtedly the fourth on account of its association with the posterior end of the cross-vein yet it occupies exactly the same place as the second light vein in the Phoridæ. This is a far more probable sort of coalescence by shortening of the cross-vein than an approximation of veins throughout their entire length which would be necessary to bring the fourth vein near the tip of the the third where the adventitious vein originates. Other points in favor of an adventitious origin are the appearance in some cases of a nebulous thickening in the same space in the Hippoboscid wing and the great variability of this vein in the Phoridæ. It also removes the difficulty which Coquillett saw in recognizing three posterior veins in the Phoridæ which he thought would exclude them from membership in the Cyclorrapha. I think also that the slight thickening near the costa beyond the tip of the third vein is also an adventitious vein if it can properly be designated as a vein.[2]

I agree with Girschner and Becker that the sixth (anal) vein is lost, or obsolete, and that the last vein (fourth light vein) represents the seventh or axillary. There can be no doubt that the short anterior branch at the tip of the third vein represents the second which is fused with it to this point. In the more specialized genera (*e. g.*, Hypocera, Puliciphora) the fusion is complete to the tip.

Public Museum Milwaukee, Wis.,
January 9, 1907.

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[1] There are many other developments among the Phoridæ which parallel those of the pupiparous Diptera such as the Hippoboscidæ. Briefly summarized they are (1) The degeneration and sometimes ultimate disappearance of the wings. (2) The production in the apterous or subapterous forms of very large eggs which distend the abdomen greatly and are even thought in some cases (cf. Wasmann,'02) to develop to the larval or pupal stage before being laid. (3) The similarity in wing venation already mentioned. (4) The degeneration of the eyes (cf. Streblidæ, Brues, 04^a, Fig. 1).

These resemblances are undoubtedly the result of parallel development, but as Professor Williston has suggested to me in a recent letter, they tend to weaken the value of the group Pupipara.

[2] Schiner refers to it as a part of the first vein.

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SOME OBSERVATIONS AND EXPERIMENTS ON THE NATURAL AND ARTIFICIAL INCUBATION OF THE EGG OF THE COMMON FOWL.

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Through a series of experiments on the developing chick, it was found that frequently the variations in development were so great that the value of the experiment was materially decreased. An attempt was therefore made to obtain more data regarding the following factors; position of eggs, turning of eggs, cooling of eggs, ventilation of eggs, moisture of eggs.

Before detailing the observations and experiments it should be emphasized that the eggs used were selected with much care. They were largely from the variety of fowls known as "Plymouth Rock." Especial care was taken to eliminate the effects of disease, inbreeding, poor food, etc. No eggs were taken from flocks in which there was not at least one cock for every ten hens. All mottled, rough shelled and ill-shaped eggs were discarded. Uniformity in size was next secured. The eggs were then tested by transmitted sunlight and those in the same stages of development selected.

Position of Eggs.

That the position of the egg during incubation has an influence on the development of the chick was shown by Dareste ('91, p. 171). It is also a well known fact among poultry raisers that the position of the egg has a profound influence upon the growth of the chick. All seem to agree that if the small end of the egg is up, the head of the chick develops in this end, and as a result, many chicks will either be deformed or fail to free themselves from the shell at the time of hatching. With these points in mind, a series of observations was made with a view of determining just what position the eggs occupy during natural incubation.

Natural Position. — Two nests containing an aggregate of twenty-seven eggs were selected which were typical of the extreme conditions. One of these was exceedingly flat, being on the ground. The other, being in a box of straw, had a very concave bottom, so that the eggs were crowded closely together; those at the periphery always rested on a sloping side.

In each of these nests, the eggs were marked so that any change of position was readily noted. A diagram of the nest was made each day, showing the position of each egg and the angle of inclination of its long axis. The sketches were made at the time the hen was feeding. If she did not leave the nest, as sometimes occurred, she was gently removed until the sketch could be made.

The angles were assigned according to the following method: an egg whose long axis formed an angle of less than 10° with the horizontal plane was marked 0°. If the long axis formed an angle of more than 10° and less than 22½° it was recorded as 15°. If the angle were more than 22½° and less than 67½° it was recorded as 45 degrees. While those whose angles were more than 67½° were recorded as 90 degrees. It was found to be impossible to register the exact angle without the expenditure of much more time than the problem merited.

In the nest with the flat bottom there was an average of less than 10 per cent. of the eggs, in which the angle of inclination exceeded 15°. From this nest of twelve eggs eleven hatched, giving a percentage of 91.7.

In the second nest which was extremely concave an average of 30 per cent. of the eggs showed an inclination of 45° or more. From this nest containing fifteen eggs, thirteen hatched, giving a percentage of 86.6. Three other nests on the ground were compared with those in straw, but in these the percentage of chicks hatched was about the same. These observations led to the conclusion that the oblique position of the egg is a factor of little or no importance in natural incubation.

It should be added that in natural incubation, one very rarely finds eggs so placed that the smaller end is uppermost. This is probably the result of two mechanical factors. Those eggs at the margin, when beneath the hen, assume an oblique position owing to the fact that this is the position in which least resistance is met; a second factor is that of specific gravity. The air space naturally tends to become uppermost and as this increases in size, the center of gravity becomes lower and lower, and the number of eggs assuming oblique positions increases as incubation progresses. It must, however, be kept in mind that an egg under natural conditions, does not maintain a position such that its angle is constant, and it may be that this varying angle of inclination influences development.

Experiments. — In order to ascertain what influence the position of the egg during artificial incubation has upon development, the following experiments were made:

In one tray of twelve-row capacity, the eggs were arranged in the following manner: Six rows were filled with eggs placed in such a position that their long axes were in a horizontal plane. Each row contained eight eggs, there being thus forty-eight eggs lying flat. In the remaining six rows the eggs were placed in such a position that their long axes coincided with an angle of 45°. Each of these rows contained ten eggs, giving sixty eggs placed obliquely. That the value of the results might not be lessened through the introduction of bad lots of eggs, they were controlled by taking equal numbers of these eggs from three different flocks of fowls and evenly distributing them throughout the tray.

On the fifth day thirteen infertile eggs were removed from those placed obliquely, leaving forty-seven living embryos. These eggs were again examined on the sixteenth day, and five dead embryos were found, leaving forty-two chicks alive at this time. Forty chicks hatched; the remaining two died after having pipped; the percentage of hatched chicks from the fertile eggs being 85.0.

From the forty-eight eggs lying flat, ten infertile eggs were removed on the fifth day, leaving thirty-eight live embryos. The sixteenth day, nine dead embryos were removed, leaving twenty-nine living chicks; of these eighteen hatched; three died after having pipped, and the remainder died in the shell. . The percentage of chicks hatched from the fertile eggs was 47.3.

It is, of course, perfectly obvious that in this experiment, the results indicated that eggs placed obliquely hatched a far higher percentage than those placed flat.

In a second experiment, the eggs were arranged in precisely the same manner, but in another incubator provided with a special ventilating apparatus. The fifth day, the eggs placed obliquely were tested and eight discarded. On the ninth day, three dead embryos were removed, and on the sixteenth day, four more were dead, leaving a total of fifty-three living eggs; forty-seven chicks hatched. Of the remaining six, four were dead in the shell, and two pipped. Thus there were hatched from those eggs placed obliquely, 88.7 per cent. of the fertile eggs.

From the forty-eight eggs placed flat, seven were found infertile on the fifth day. On the ninth day, six dead embryos were removed. On the sixteenth day, but one dead embryo was found. Thirty four chicks hatched. There was hatched about 83 per cent. of the fertile eggs.

The results of the foregoing observations and experiments taken together, lead to the deduction that when the supply of fresh air (oxygen) is inadequate, the oblique position of the egg, thereby bringing the embryo in closer contact with the air chamber, is decidedly advantageous. When there is an abundant supply of fresh air, there is but little to be gained through placing the eggs obliquely.

Turning of Eggs.

How many times the hen turns her eggs during the time of natural incubation is a question often asked, but as yet unanswered. Reaumer ('49, p. 166) states that they are turned daily and Dareste ('91, p. 161) that they are often turned twice daily. Believing this to be a point well worthy of investigation, a series of observations was made with the hope of obtaining some information as to the influence this factor plays in development.

Experiments have previously been made with a view of ascertaining what influence turning the egg has upon development. Dareste ('91, p. 165) placed sixteen eggs under the same conditions of artificial incubation. Eight were unmoved, while the remaining eight were turned twice each day. In the first set absorption of the yolk did not occur in any case; the embryos died during the second or third week. Of the eight which were turned six developed normally; a seventh was opened on the twenty-second day, showing normal conditions; while in the eighth the chick died on the twentieth day, the adhesions between the allantois and the yolk having prevented the absorption of the latter.

Natural Turning. — In order to obtain satisfactory data, it was necessary to arrange nests in such a manner that the position of each egg could be sketched at frequent intervals without disturbing the hen. Three nests with felt sides and concave glass bottoms were constructed, and placed in such positions that the eggs could be viewed and sketched from below. The eggs were numbered in four places about equally distant from each other and midway between the two ends of the egg. Each figure bore an alphabetical index. The numbers on egg 1 would run thus: 1*a*, 1*b*, 1*c*, 1*d*, so that any degree of turning might be readily observed. Five sketches or plats of each nest were made each day during the incubating period, and at the following hours: 6 A. M., 9 A. M., 12 M., 3 P. M., 6 P. M.

The observations show that the eggs are turned partially or completely much more frequently than has been supposed, at least five times during any given day. It should also be stated that on a number of days sketches were made at more frequent intervals, and in nearly every case the eggs had been partially or completely rotated. The observations made were confined to the twelve hours between 6 A. M. and 6 P. M. A number of scattered observations lead to the belief that the eggs are also turned during the night, but just how frequently is yet to be determined.

The hen turns the eggs in two ways. If a sitting hen be watched as she returns from feeding to sitting, it will be seen that she moves her body rapidly from side to side. Whether the object be to turn the eggs is uncertain. Probably the first object is to bring the surface of the body in the closest possible contact with the growing embryos. Accidentally or purposely, she also turns the eggs. This is not only true of the hen returning from feeding, but also when on the nest, for she is frequently observed moving about and settling down with the same characteristic lateral movements. Sometimes there are so many eggs in the nest or they are so widely scattered that the hen fails to properly cover them. When such conditions occur, the hen invariably uses her beak to bring the outlying eggs in contact with her body. Not only does she frequently thus turn the eggs, but also she very often reaches beneath her body and turns the eggs lying near the center of the nest. Why she does this is a question which awaits an answer.

Experiments. — Seventy-five eggs were selected from three lots of fowls, arranged in three groups and so placed that the eggs from one lot of fowls alternated with those from another lot. Those eggs of group I. were left unmoved; those of group II. were turned at 6 A. M., and 6 P. M.; those of group III. were turned at 6 A. M., 9 A. M., 12 M., 3 P. M. and 6 P. M.

From group I., five infertile eggs were removed on the fifth day, together with eight dead embryos, six of which had grown fast to the shell membrane. The eggs were again examined on the twelfth day and five more dead embryos were removed. These were examined and four were found to have the allantois grown fast to the yolk; but three chicks hatched from the entire twenty-five. The remaining four were dead in the shell. The number hatched was 15 per cent. of the fertile eggs.

In group II. there were three infertile eggs removed on the fifth day, and one dead embryo which had adhered to the shell membrane. On the twelfth day four dead embryos were removed, one had the allantois adhering to the yoke. In the others the cause of death could only be surmised. From the remainder, ten chicks were hatched; the others died in the shell, giving a hatch of 45.4 per cent. of the fertile eggs.

In group III. six infertile eggs were found on the fifth day and no dead embryos. On the twelfth day one dead embryo was found. Eleven chicks hatched; two died after having pipped, while the remaining five were dead in the shell; the number of hatched chicks being 58 per cent. of the fertile eggs. The experiments indicate that frequent turnings (at least five) give best results.

It is necessary to point out, however, that a very low percentage of chicks hatched even in group III. This is to be attributed directly to a lack of sufficient oxygen, the incubator in which the experiments were made being poorly ventilated, owing to the fact that the ventilating system had been modified in order to compare its results with those provided with special ventilation. A second incubator provided with a special ventilating apparatus, hatched 83 per cent. and 88.6 per cent. of two lots of eggs taken from the same fowls.

Dareste concluded from his experiments that during the first week of artificial incubation, eggs which are unturned develop in essentially the same manner as those which are turned. The principal cause of death is due to the allantois growing fast to the yolk, causing the rupture of the vitelline membrane, thereby allowing the yolk to escape so that it cannot be taken into the body of the embryo. Dareste adds that when the eggs are turned it is probable that the position of the allantois is shifted, and this movement prevents its adhesion to the yolk. It should be remarked that during the early days of incubation it is also necessary to turn the eggs frequently ; otherwise, the embryo grows fast to the shell membrane. This has not only been shown by the preceding experiments, but has been repeatedly observed in other eggs.

IV. Temperature of Eggs.

Repeated attempts have been made to ascertain the temperature of the egg during natural incubation, but as yet the results are far from satisfactory. This is due to the difficulty experienced in testing the temperature of different parts of the egg. The fact that the egg comes in contact with a heating surface above and a cooling surface below, leads to most perplexing complications. While the temperature of the hen is easily ascertained, it is not an easy matter to know the precise degree of heat applied to the surface of the egg. Moreover, it should be kept in mind that during incubation, not all the eggs are at all times in contact with the body of the hen. A layer of feathers intervenes to modify the temperature, this layer varying greatly in thickness in different parts of the body, and at different times during incubation. All these factors conspire to make an exceedingly difficult problem.

Natural Temperature of Hen. — In attempting to determine the daily temperature of the hen, special self-registering thermometers were fastened to blocks so cut that their upper surfaces were nearly egg-shaped. The lower surfaces of the blocks were broad and flat, so that they could not be easily overturned. One was placed in each of four nests and left for two or three hours, when the reading was made.

The following table shows the temperatures obtained by this method, during twenty days of incubation. In this as in subsequent tables, the Roman numerals indicate the serial numbers by which the hens were designated, while those above the columns indicate the day of incubation.

	1	2	3	4	5	6	7	8	9	10
I.	102.1	103.0	103.0	103.8	105.0	104.5	105.0	105.0	106.2	106.0
II.	103.0	104.0	103.5	104.5	104.5	104.0	105.0	105.5	104.5	104.6
III.	102.0	102.0	103.0	103.0	105.0	105.0	104.5	104.0	104.5	104.0
IV.	101.5	102.5	102.5	103.0	103.5	104.0	104.5	104.5	105.0	105.0
	11	12	13	14	15	16	17	18	19	20
I.	105.0	104.5	105.0	105.5	104.5	105.5	104.8	105.0	104.5	105.5
II.	104.6	104.5	104.6	104.2	105.0	104.8	105.0	105.0	105.0	104.0
III.	104.0	105.0	104.0	103.6	104.0	105.2	104.2	103.5	103.0	104.0
IV.	104.8	105.0	104.5	105.0	104.8	105.0	105.0	104.5	104.5	105.0

A second series of readings was made by gently removing the hen from the nest and placing the thermometer in the groin for five minutes. The results are of course, somewhat unsatisfactory, since the excitement of the fowls due to their being removed from the nest, results in a temperature somewhat higher than the normal.

	1	2	3	4	5	6	7	8	9	10
I.	103.0	104.0	103.5	104.6	105.5	105.0	106.0	106.0	105.5	106.0
II.	104.0	105.2	105.5	105.5	105.5	106.5	105.2	106.0	106.0	107.0
III.	103.5	103.2	105.5	106.5	106.2	105.0	105.2	105.0	105.0	105.0
IV.	102.0	102.5	104.0	104.5	105.0	105.0	104.5	105.0	105.0	105.5
	11	12	13	14	15	16	17	18	19	20
I.	106.5	105.8	105.6	105.5	106.2	106.2	106.0	105.5	105.8	105.5
II.	106.2	106.5	106.5	106.0	107.4	106.0	106.0	106.5	106.2	106.5
III.	105.8	105.0	105.2	106.2	106.5	106.0	106.5	106.0	106.2	106.0
IV.	105.0	105.2	105.6	106.0	105.5	105.2	105.0	105.0	104.8	105.0

Natural Temperature of Egg. — Since experiments show that the above temperatures are too high for artificial incubation, it is necessary to push the inquiry a step further with a view of determining the exact temperature of the egg during natural incubation. The temperature of the hen recorded below was obtained from a thermometer attached to a block as described above. The temperature of the egg was taken in the following manner: A pail of lukewarm water was brought to a temperature of 98° F. (by the addition of warm or cold water). The egg was then placed in a tightly fitted rubber bag and held about four inches below the surface of the water. An opening was then made in the shell directly over the embryo, and a self registering thermometer warmed to 98° inserted for five minutes. The thermometer was inserted just far enough to bring its lower end at the center of the egg. As often as the eggs were broken for testing, they were replaced by eggs taken from other hens set at the same time.

	1	2	3	4	5	6	7	8	9	10
Hen.	102.2	103.0	103.5	104.0	103.8	105.0	104.6	104.5	105.0	105.0
Egg.	98.0	100.2	100.5	100.5	100.4	101.0	101.8	102.5	101.6	102.0
	11	12	13	14	15	16	17	18	19	20
Hen.	104.8	105.2	104.5	105.0	105.2	105.0	104.6	104.8	104 5	104.5
Egg.	101.8	102.2	102.0	102.5	102.0	103.0	102.4	103.0	103.0	103.0

A second series of readings was made by gently removing the hen from the nest and placing the thermometer in the groin for five minutes. The results are of course somewhat unsatisfactory since again the excitement of the fowls, due to their being removed from the nests, resulted in temperatures somewhat higher than normal.

	1	2	3	4	5	6	7	8	9	10
Hen.	103.0	105.0	104.8	104.2	105.2	105.0	104.8	104.8	105.0	105.0
Egg.	99.5	100.0	100.2	100.5	100.6	101.0	100.5	100.5	101.5	101.5
	11	12	13	14	15	16	17	18	19	20
Hen.	104.8	105.0	104.8	104.8	105.2	105.0	105.5	104.0	104.0	104.0
Egg.	101.5	101 2	100.8	101.8	102.0	101.8	102.2	102.0	102.4	102.4

While the above are the only sets of daily observations, they were supplemented by a number of scattered tests. In no case was the egg found to exceed the temperature given in the table by more than one degree. In but few cases was it found to be a degree lower. Although it cannot positively be stated that these tested eggs would have hatched, the inference seems more than probable, since in five other cases where hens were set on eggs from the same flocks of fowls, the fertile eggs hatched with but very few exceptions. These observations show that the proper incubating temperature of the egg is about 100° for the first week; 101° for the second, and 102°–103° for the final week.

Temperature of Artificially Incubated Eggs. — The next problem is to determine what temperature must be kept in the air chamber of the incubator in order to obtain the above temperature of the egg. A series of observations was made on the artificially incubated egg. The temperature of the egg chamber was read from a thermometer placed flat and on a level with the top of the eggs, but not in contact with them. The temperatures of the eggs were taken in precisely the same manner as in the preceding experiment.

	1	2	3	4	5	6	7	8	9	10
Inc'b.	103.0	103.5	103.0	104.0	103.5	104.0	103.0	103.5	105.0	103.0
Egg.	100.2	100.0	100.0	101.2	101.6	101.8	101.6	100.0	102.8	101.0

	11	12	13	14	15	16	17	18	19	20
Inc'b.	103.0	104.0	105.0	103.0	104.5	103.0	105.0	104.5	106.0	106.0
Egg.	102.0	102.6	103.7	102.5	103.6	104.8	104.0	104.0	—	—

The above record was made from an incubator which hatched about 85 per cent. of the fertile eggs. The hatch, however, was somewhat premature, since many of the eggs hatched on the nineteenth day. It is thus evident that a temperature somewhat too high had been carried. In view of the irregularities of the incubator a second experiment was made.

	1	2	3	4	5	6	7	8	9	10
Inc'b.	102.0	102.0	103.0	102.0	102.5	103.0	102.5	102.0	103.0	103.5
Egg.	99.5	100.0	101.0	100.5	100.5	101.0	100.0	100.0	101.0	101.5

	11	12	13	14	15	16	17	18	19	20
Inc'b.	103.0	103.5	104.0	103.5	104.0	104.5	104.0	103.5	104.0	104.5
Egg.	101.5	101.8	102.0	102.5	103.0	103.0	103.0	102.5	102.5	103.5

As will be observed, the incubator was under better control in the second experiment and the chicks hatched on the twentieth and twenty-first days yet the percentage hatched was about the same as in the first, not including in either case the eggs destroyed in making the tests. While a more extended series of tests would be highly desirable, one certainly does not widely err in stating that the most favorable temperature within the egg chamber is close to 102°–103° F. the first half of the incubating period and 103°–104° F. for the latter half.

In any consideration of temperature, the fact must be kept in mind that as the chick grows, it gives off more and more heat, so that if an incubator of 200 egg capacity were entirely without artificial heating, the temperature would be much higher than that of the surrounding atmosphere; it consequently follows that less artificial heat is necessary during the later stages of incubation. The 102°–103° in the earlier stages is largely artificial heat, while the 103°–104° in the later stages would be the combined animal heat, given off by the egg, and the artificial heat supplied by the heat radiator.

V. Cooling of Eggs.

How frequently the eggs should be cooled and for how long a period, is a question of considerable importance. Not being entirely satisfied with the data at hand, an attempt was made to gather some information by actually watching a number of hens from day to day.

Natural Exposure. — Six hens were observed throughout the period of incubation and the results tabulated. It was found that the average time the hen leaves her nest, during the first fifteen or eighteen days is about thirty minutes. During the last few days of the incubating period she rarely leaves the nest. The longest time a nest was left exposed was an hour and twenty minutes, and the shortest time about twelve minutes. If the hen be obliged to forage for food, she remains a much longer time than when food is at hand. But one or two instances were noted in which the eggs were exposed for much more than an hour. It may be stated with a fair degree of certainty that the cooling of the eggs is due to the necessity of obtaining food, and in no way fundamentally affects the growth of the chick when there is an abundant supply of fresh air. There is not the least doubt, however, but what it has a beneficial influence in cases of poor ventilation, and since no incubator is supplied with too much, it probably is best to adopt the common practice of cooling the eggs. In so doing it would not seem advisable to cool the eggs for more than twenty to thirty minutes each day, for the first fifteen or eighteen days.

VI. Ventilation of Eggs.

Natural Ventilation. — In natural incubation a perfect ventilation exists. An abundance of fresh air can always reach the eggs by diffusing through the feathers which cover them. This process is constantly going on during incubation, the foul air likewise having free exit. There is thus ample opportunity for a continuous circulation of air, and there is every reason to believe that it takes place. There is also afforded by the feathers a complete barrier against sudden draughts of air. The fresh air is also raised to a certain temperature through the heat of the hen before it comes in contact with the eggs, which also serves to reduce any excessive humidity. This perfect system of ventilation cannot fail to impress one of its importance in facilitating the growth of the chick.

Dareste ('91, p. 150) conducted the following series of experiments: All the apertures of the incubator were closed during incubation, and upon examination it was found that nearly all the embryos had died. It was found further, that there had developed in the albumen a microscopic organism resembling the ordinary yeast plant. The author concludes that air modified by embryonic respiration, facilitates the growth of parasitic organisms.

Gerlach ('82, p. 115) found that by diminishing the quantity of air during incubation, he could cause dwarfing of the embryo. He then tried whether an increase in the size of the embryo could be brought about by increasing the quantity of air. A part of the shell was scraped very thin and placed in an incubator. During the first two days the normal and modified eggs were alike, but after that time the embryo in the scraped eggs developed at a remarkably rapid rate, nearly twice as fast as in normal growth.

A second method of increasing the supply of air was to remove whole pieces of the shell. Of course great care was taken not to injure the shell membrane or growing blastoderm. This fracture was made some distance from the embryo, so that the drying could not extend to the embryo, and the egg after the removal of the part of the shell was turned so that the broken portion was downward. The embryo was perfectly formed, but grew at the same astonishingly rapid rate.

The above consideration led to the conviction that artificial incubation can only proceed where there is an abundant supply of fresh air (oxygen). In order to confirm this supposition, the following experiment was tried.

Experiments. — Two incubators with similar ventilating systems, which, however, were believed to be inadequate, were employed. One was left with the ventilating system unmodified. The other was provided with two one-inch intake pipes. These extended to the outside of the building in which the incubators were located, and so arranged that a continuous current of fresh air passed into the egg-chamber.

Two egg trays of 100 capacity each, were filled with eggs from the same lots of fowls ; special care being taken to divide the eggs from each flock so that there should be an equal number in each tray. The eggs were then subjected to exactly the same treatment, barring slight variations in temperature which necessarily existed.

On the fifth day the eggs were tested, and from the incubator with special ventilation, sixteen (infertile) eggs were removed. From the other, twelve (infertile) eggs were removed. On the twelfth day they were again examined. From the incubator with special ventilation seven dead embryos were removed and from the other, twenty. From the eighty-four in the incubator with special ventilation, seventy-two hatched, while five were dead in the shell, giving a percentage of 85.7 per cent. hatched from the fertile eggs. Of the eighty-eight eggs remaining in the other incubator, but thirty-nine hatched ; a number of the remainder pipped, just how many was not recorded, while a large number were dead in the shell. There was thus hatched in the incubator without ventilation 44.3 per cent. of the fertile eggs.

But when a perfect ventilation has been obtained, it has produced certain deleterious effects which must be corrected. It is commonplace to say that when evaportion goes on in still air, this air soon becomes saturated, and evaporation, if not stopped, goes on very slowly. If, however, the saturated air is constantly removed and dry air takes its place, the rate of evaporation is increased. It is thus evident that any discussion of ventilation must take into consideration the question of moisture.

VII. Moisture of Eggs.

There is probably no one factor so little understood as that of moisture. The most careful observations of the nesting habits of the hen seem to only complicate the matter. A hen may build her nest on the ground, or in the hay loft, and in each case hatch about the same percentage. These facts, which are a matter of every-day observation, lead us to believe that eggs hatch equally well under these variable conditions. The moisture necessary for development must then be controlled by the hen, or egg, or both.

There are certain constant factors in the production of moisture which we may accept as existing. First of all, the temperature of the air in the nest is far higher than that of the outside air. As the two come in contact, there is more or less moisture produced. A second source is from the perspiration of the skin. A third source is from the egg itself. These three sources supply, so far as we are able to determine, the moisture necessary for the normal development of the egg.

It is known through the experiment of Reaumer, that excessive moisture gives rise to the pathological forms. Dareste (p. 159) also records an experiment in which the atmosphere was saturated and as a result the albumen liquified and leaked through the shell. Furthermore, Dareste, stated that excessive moisture facilitates the growth of parasitic forms which develop in the albumen.

The writer made a series of daily hygrometer tests with a view of ascertaining just how much moisture existed in the nests. In testing with the hygrometer, it was placed in the nest among the eggs, and at the end of fifteen minutes was taken out and the reading recorded. Although a great number of these tests were made and tabulated they were later discarded, owing to wide variations in the hygrometers and the problem attacked in another way. It is of course well known that the egg decreases in weight during incubation and that this is due chiefly to the evaporation. In order to find out definitely how much evaporation goes on during natural incubation, thirty-six eggs were weighed each day for twenty days and these weighings tabulated. It was found on the average that the egg during natural incubation loses about 13 per cent. of its original weight.

It was also found by experiment that the evaporation could be lessened until the egg lost but 9 per cent of its original weight and still give a healthy chick. It was likewise learned that evaporation could be increased up to about 20 per cent and the eggs give rise to perfect chicks. It would thus appear that the moisture in the incubator should be so controlled that it will allow the evaporation of about 13 per cent of the original weight of the egg.

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'91 Production artificielle des monstruosities. Paris, 1891.
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'82 Doppelmisbildungen bei den höheren Wirbelthieren. 1882.
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'49 Art de faire éclore d'eleve en toute saison des oiseaux domestique de toutes espèces, soit par le moyen de la chaleur des furnier, soit par le moyen de celle du feu ordinaire. Paris, 1849.

A PECULIAR PELVIC ATTACHMENT IN NECTURUS MACULATUS.

CLARA HEPBURN.

While studying the vertebral column of *Necturus maculatus*, in the regular class work, I found in my specimens a peculiar form of pelvic attachment which seems remarkable enough to mention.

The pelvis was attached to the eighteenth vertebra and, in addition, there were two rudimentary ribs on the nineteenth. There was nothing strange about the twentieth but on the twenty-first there was one half of a hæmal spine, that of the right side, the first complete hæmal arch being on the twenty-second.

For comparison in order to show its anomalous character, it is necessary to give the statistics concerning the conditions as reported hitherto. F. Smith, '00, and Wilder, '02, have summarized the results hitherto reported, including the papers of Parker, '96, Bumpus, '97, and Waite, '97.

In one hundred and fourteen specimens tabulated by Smith, the sacral vertebra was the nineteenth in eighty-one cases, the twentieth in sixteen and the twenty-first in one, this latter being the most posterior position ever recorded. In twelve cases the pelvis was oblique and of these three made use of the eighteenth on one side. In four cases there were three sacral ribs, that is, two upon one side and in one of these, figured by Smith on page 638, the ribs involved were the eighteenth on the right and the eighteenth and the nineteenth on the left. Thus in Smith's one hundred and fourteen cases, four involved the eighteenth vertebra but all were asymmetrical. The one which approached nearest my specimen is the one figured by Smith, where there was a ligamentous connection between the rib rudiment on the nineteenth and the sacrum, and as my specimen had been macerated before I examined it, I cannot tell whether there was such a connection or not.

As Wilder has used the same authors for his summary as has Smith, it is unnecessary to give his report.

None of these authors mention a case with the symmetrical pelvic attachment of both sides upon the eighteenth vertebra, nor one with symmetrical rudimentary ribs on any vertebra posterior to the sacral connection. It is rather significant that the nineteenth vertebra, the one that in the majority of cases bears the sacral ribs, should, in my specimen, have borne the rudimentary ones.

The fact that in this particular case, the first entire hæmal arch was found on the twenty-second vertebra, is not so remarkable. F. Smith records this position in sixty-two cases out of two hundred and forty-one. Wilder, also, states that the hæmal arch appears suddenly on the twenty-second to the twenty-fourth vertebra, usually the twenty-third. He then adds that in one case the vertebra just anterior to the first one bearing a complete hæmal arch, bore upon one side a slender process, four to five millimeters long, evidently representing an incomplete hæmal arch. But whether the vertebra which bore the complete hæmal arch was the twenty-second, twenty-third or twenty-fourth he does not say, so that one cannot tell whether the vertebra with the incomplete hæmal arch was the twenty-first, as in my specimen, or one of the two next posterior.

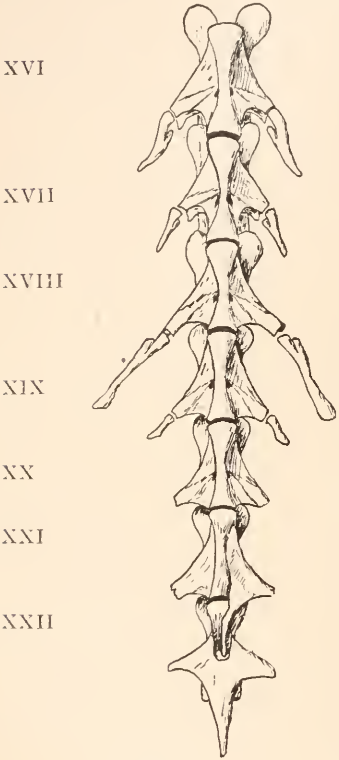

Fig. 1.

Although Bumpus has shown by a careful comparison that the variation of the position of the first hæmal arch is entirely independent of that of the sacral vertebra yet the presence of even a partial arch upon a vertebra anterior to any previously recorded, when taken in connection with the extreme anterior location of the sacral connection, appears important.

Summarizing, then, the points of especial interest in the case in question, we have the following:

1. The pelvic attachment to the eighteenth vertebra on both sides.

2. The presence of a pair of rudimentary ribs upon the nineteenth vertebra, the position of the usual pelvic attachment.

3. The presence of an incomplete hæmal arch upon the twenty-first vertebra.

Smith College,
December 11, 1906.

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NOTE ON THE CONUS ARTERIOSUS OF MEGALOPS CYPRINOIDES (BROUSSONET).

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Since describing the conus arteriosus in *Tarpon Atlanticus*[1] I have been fortunate in securing a specimen of *Magalops cyprinoides.* For this I take the present opportunity of thanking Professor David Starr Jordan.

The fish in question, preserved in alcohol, measures 19 cm. (including caudal fin) so that the heart is extremely small, and is, on account of its somewhat friable condition, difficult to handle.

The conus is everywhere quite obvious from the exterior. Fig. 1, drawn from the left side, indicates that the general form of conus and bulbus resembles that of *Amia* rather than that of *Tarpon.*

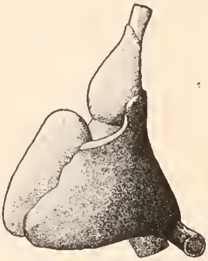

FIG. 1.

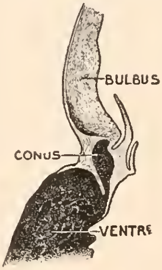

FIG. 2.

FIG. 1. Entire heart of *Megalops cyprinoides* from the left side, × 3. A small portion of the atrium has been removed to display the conus more fully.

FIG. 2. Frontal section through the right side of the heart of *Megalops cyprinoides*, showing the relations in the conus region, × 10.

The heart was opened by a mid-ventral sagittal incision, it having been previously ascertained that such an incision would fall between the valves without cutting them. After examination and measurement the two halves were imbedded in celloidin and cut into serial sections.

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[1] BIOL. BULL., February, 1907, p. 145. (The literature on the conus arteriosus is given in this article.)

The extreme length of conus is 1 mm. in the mid-ventral line and 1.5 mm. in the mid-dorsal and lateral lines. The ventricle, from apex to junction with conus, has a mean measurement of 5.5 mm. The proportion in mean length of conus to ventricle is therefore 1 to 4.

The conus contains two transverse tiers of valves, each tier consisting of a right and left cusp placed symmetrically with regard to the mid-sagittal plane. The general arrangement agrees closely with that found in *Tarpon*, but the proximal cusps appear to be proportionately more capacious.

The conus in *Megalops* not only projects more freely from the ventricle than in *Tarpon*, but is of greater proportionate length. It would seem to resemble more closely the conus of *Albula* (as described by Boas, '80) except in the absence of the subsidiary valve cusps of the latter.

It should be noticed that the heart described is from a young fish, also that the measurements are, at best, approximate; therefore, comparisons with adults of other genera, if pushed too closely, are apt to be misleading.

The atrio-ventricular valve has three cusps.

A specimen of *Chanos chanos* (Forskål), for which I also have to thank Professor Jordan, presents an easily recognizable vestigial conus arteriosus, but only one tier of valves.

St. Louis, Mo., February 1, 1907.

SEX DIFFERENTIATION IN LARVAL INSECTS.

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The question of the causes of sex differentiation is a problem to the solution of which we seem now to be only a little nearer, despite numerous researches, than we were many years ago. It is advisable, perhaps, to continue to attempt to overcome some of the outworks of this well entrenched problem. One of the outliers of the main problem may be described in the phrase "When is sex differentiated?", another in the phrase "Does nutrition affect sex?"

Being engaged in rearing experimentally large numbers of silkworm moths, *Bombyx mori*, I have taken advantage of the opportunity to test for this species both these subsidiary parts of the sex differentiation problem. Various lots of larvæ were set apart, each individual being isolated so as to insure identity of nutrition conditions, and fed on short rations. The result of these experiments is given in a paper in the *Journal of Experimental Zoology* (vol. 1, pp. 357–360, 1904). It is sufficient

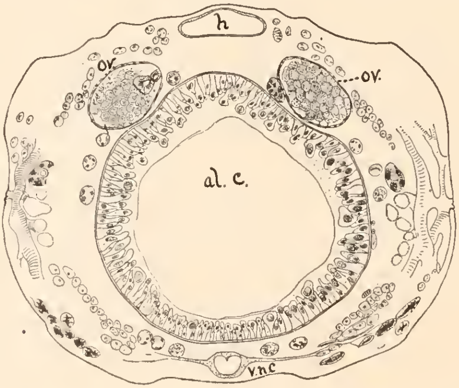

Fig. 1. Section through female *Bombyx* larva, just after the third moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord.

to say here, in a word, that this "short feeding" produced no apparent effect in determining the sex of the moths.

Since these experiments I have learned a good reason why the nutrition of the larva, at least after its first moulting, should have no effect on the sex as revealed in the moth, and this reason is that the sex of each individual is definitively determined at least as early as immediately after the first larval moulting, as may be readily perceived by an examination of the rudimentary reproductive glands.

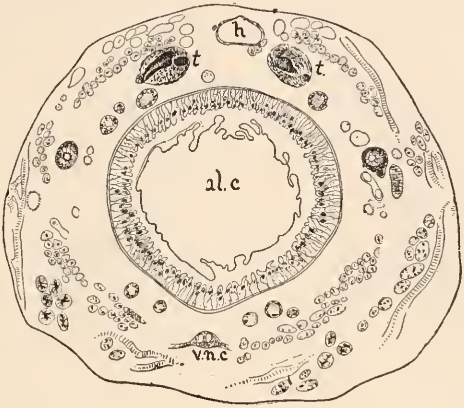

FIG. 2. Section through male *Bombyx* larva, just before the third moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *t*, *t*, testes.

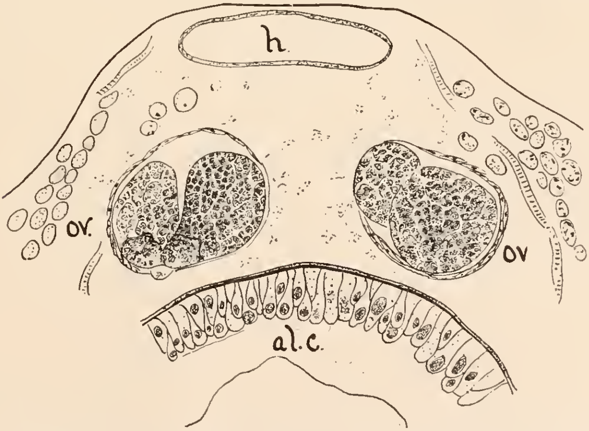

FIG. 3. Section (dorsal third) through female *Bombyx* larva, just after the second moult; *h*, heart; *al. c*, alimentary canal; *ov*, *ov*, ovaries.

In the larval silkworm there may be noted on the dorsal wall of the fifth abdominal segment two low tubercles, rather dark colored. Directly beneath these spots lie the developing reproductive organs (ovaries, testes). By dissecting a number of larvæ in their last or next to last intermoulting period, a marked difference will be noted in the size of the organs in different individuals. By dissecting out the organs and sectioning them, constant marked histologic differences will be seen in the two sizes of organs. As a matter of fact the larger are ovaries, with well-developed rudiments of egg-tubes, the larvæ possessing

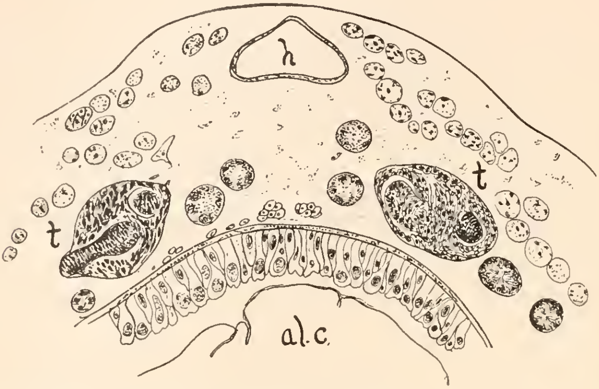

FIG. 4. Section (dorsal third) through male *Bombyx* larva, just after the second moult; *h*, heart; *al. c*, alimentary canal; *t*, *t*, testes.

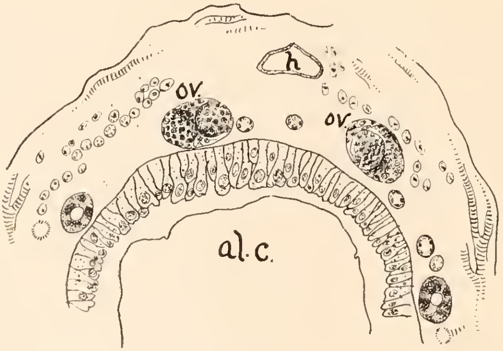

FIG. 5. Section (dorsal half) through female *Bombyx* larva, just after the first moult; *h*, heart; *al. c*, alimentary canal; *ov*, *ov*, ovaries.

them being female individuals, while the smaller are testes. By dissecting larvæ of successively younger age, these differences in size and histologic character may be followed back to just after the first moulting.

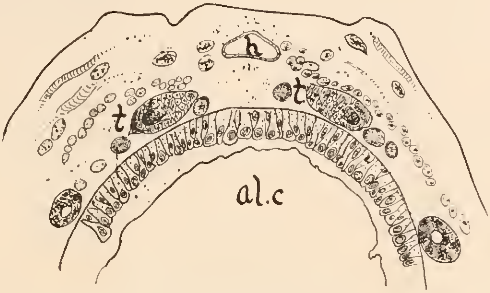

FIG. 6. Section (dorsal half) through male *Bombyx* larva, just after the first moult; *h*, heart; *al. c*, alimentary canal; *t*, *t*, testes.

Figs. 1 to 8 show, with little need of explanatory description, the gross character of the histologic differences, the relative sizes (in cross-section) of the two kinds of organs, and also the relative size of these organs in the various larval stages. I have not been sure of being able to distinguish between the sizes or the histologic characters of the reproductive organs in the just

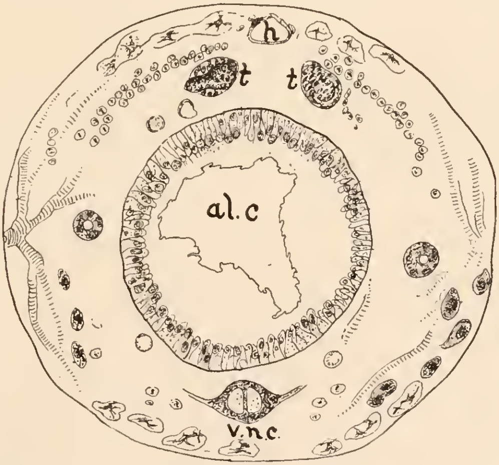

FIG. 7. Section through male *Bombyx* larva, just before the first moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *t*, *t*, testes.

hatched larvæ, but even here the organs are conspicuous and well started in development, being larger in diameter than the heart or the spinning glands.

We may affirm then positively that from the time of the first moulting on the silkworm larva has its sex determined: its reproductive glands are ovaries or testes distinguishable by obvious histologic characteristics. Also that the just hatched larva has reproductive organs already well developed. I have little doubt that careful scrutiny of the organs at this stage would reveal to the trained histologist, especially to the student of oögenesis and spermatogenesis, definitive characteristics marking sex differentiation. The sex of the silkworm is not to be tampered with by gorging or starving, and what is true of this lepidopteron is undoubtedly true of its cousins, the other moths and the butterflies. It is probably also true of other insects with complete metamorphosis. I recall dissections of various larvæ, notably of *Corydalis cornuta* (a neuropteron) and of *Holorusia rubiginosa* (a dipteron) in which the reproductive organs appear of two sizes in specimens of the same age: indeed in *Corydalis,* of two shapes. These organs need histologic examination. Some student should laboriously work through a long and representative series of insects and settle the question as to the time of sex differentiation. That is, find out whether it be true for all, as it is in the silkworm, that the time of sex differentiation is obvious before, or, at latest, at very little after the time of hatching. If it is true, the question of the influence of nutrition in sex determination will also be settled — for insects. And we need waste no more time in tedious feeding and tabulating.

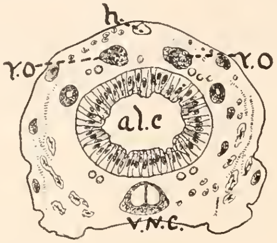

FIG. 8. Section through *Bombyx* larva (male or female?), two days after hatching; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *r*, *o*, reproductive organs.

FURTHER NOTES ON THE REGENERATION OF THE FINS OF FUNDULUS HETEROCLITUS.

G. G. SCOTT.

During the last summer the writer while engaged as assistant at the biological laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass., conducted various experiments on *Fundulus heteroclitus.* Since a number of these can be conveniently grouped under the heading *regeneration* they are collected and presented in the present paper. Various factors determining regeneration have been under discussion and it was thought that these should be tested using *Fundulus h.* as a type since it is a representative teleost and that therefore results might differ from those obtained in crustaceans, for example, and that certain principles deduced from crustacean work might possibly have to be modified. Thanks are due to Dr. F. B. Sumner as director of the laboratory of the Bureau of Fisheries for assistance in providing the necessary apparatus and other facilities.

The following experiments were designed to show: (*a*) the relation of temperature to regeneration, (*b*) the relation of degree of injury to regeneration, (*c*) the relation of length and weight (*i. e.*, age) of specimen to regeneration, (*d*) the relation of amount of food to regeneration, (*e*) adaptation in regeneration.

(A) RELATION OF TEMPERATURE TO REGENERATION.

With a view of testing this the caudal fin of ten *Fundulus h.* were cut in a line as near as possible to the origin of the caudal fin rays. The same procedure was followed in all the later cases to be described. These fish were placed in a chest or jar with three liters of sea water and the jar placed in an ice chest where the temperature averaged 14°C. The water was changed every day or so. On August 24 five fish had survived. The fish had not been fed. The survivors had been under observations for 41 days or almost six weeks. But in those survivors there was no sign of regeneration. With fishes in normal conditions regeneration is evident after the first few days after operation. In order to further test this point and ascertain whether lack of food had anything to do with the result, two lots of fishes were operated on in a similar manner on September 1. Each lot was placed in a jar in three liters of sea water and both jars placed in the ice chest. One lot was fed and the other not fed. On September 14 nine tenths of those that had not been fed had survived and seven tenths of the others. There was no sign of regeneration in either lot. It might be added that in case of another experiment set up at the same time that regeneration was evident a few days after. (See Table III.)

It may be of interest to note that two of the survivors mentioned in the first experiment were placed in a shallow jar surrounded by running sea water, and in two weeks time the caudal fin showed a regeneration of 2 mm. indicating that just as soon as the temperature was raised to the normal regeneration began. It should also be noted that the temperature necessary to inhibit regeneration was not an extremely low one, and that this indicates that the power of regeneration is sensitive to lowering of temperature.

(B) Relation of Amount of Injury to Regeneration.

In 1902 Zeleny observed that when two chelæ of *Gelasimus*, the fiddler crab, were removed each of the regenerating buds grows more rapidly than does the single one when only one chela is removed. In 1903 the same author found that the regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the number of arms removed, *i. e.*, that animals with the greater number of arms removed regenerate more rapidly. The greater the degree of injury the more rapid the regeneration. In 1905 Zeleny in an experiment extending over 181 days using the common cray-fish, *Cambarus propinquus*, found that in the series with the greater degree of injury (Series B) each chela regenerates more rapidly than the single chela of those with the lesser degree of injury (Series A). in Series A consisting of 36 individuals the right chela was removed at its breaking joint. In series B consisting of 41 individuals the two chelæ and the last two pairs of walking legs were similarly removed. It is to be regretted that the same author did not include a third series in which the injury was midway between that in A and B to see whether regeneration in that third series would be intermediate between that of series A and B. Thus Zeleny shows that in the cray-fish the greater degree of injury has a stimulating effect on regeneration.

On the other hand Emmel ('04) removed the right cheliped in the lobster immediately after moulting and allowed it to regenerate for the period between moults then removed it again after that moult and allowed it to regenerate. He found that it regenerated less between moults the more it was mutilated. In other words Emmel finds in a general way in a nearly allied form just the opposite result to Zeleny. In 1906 Emmel found a decrease in rate of moulting as correlated with greater degree of injury and lesser rate of regeneration, again a contradictory result to that of Zeleny. With a view of testing this question the following experiment was devised. Three lots of fishes were taken designated as *A*, *B*, *C*.

In *A* the caudal fin was amputated.

In *B* the caudal and one pectoral.

In *C* the caudal and both pectorals.

The fishes were kept in separate compartments in the hatchery with running sea water but were not fed. The experiment was begun on July 30 and continued until September 4 when all were removed and measured with fine pointed registering calipers. In the following Table I. are found the results of these measurements. Not only is the length of the specimen given but the amount of regeneration and the specific regeneration. This is a term used by Zeleny. Since the specimens differ in length we must devise some means of comparing regeneration in one case with that in another. We can do this by finding the percentage regeneration in each case, *i. e.*, by dividing the amount of regeneration by the length of the specimen. We may use Zeleny's term and call it specific regeneration. In the table is also given the weight of each specimen for purposes to be described later.

Now in interpreting these results we have recourse to the statistical method. We should apply certain formulæ here to ascertain whether differences in resulting regeneration in the three different lots are great enough to ascribe any importance to them and thus say that they are due to difference in degree of injury—or on the other hand whether after all they are not simply chance discrepancies. Workers in statistical method have deduced the law that if the mean difference of one character in any two series of forms is less than the probable errors of the difference then the

Table I.

Lot A.

	Length Sp.	Amt Reg. Caudal.	Sp. Reg.	Wt. Sp.
1	8.1 cm.	.55 cm.	.0676	6.27 gm.
2	8.0	.65	.0813	5.80
3	6.5	.60	.0923	3.05
4	6.35	.80	.1259	3.38
5	7.25	.65	.0896	4.20
6	9.2	.65	.0706	11.55
7	10.2	.75	.0736	16.70
8	8.25	.80	.0969	8.4
9	9.4	.70	.0744	7.5
10	7.9	.60	.0760	6.25
11	6.35	.60	.0944	3.9
12	8.4	.80	.0952	10.12
13	8.4	.60	.0714	8.85

Lot B.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	11.3	.70	.0610	.55	.0486	26.87
2	7.1	.65	.0915	.50	.0704	4.75
3	8.3	.70	.0843	.60	.0723	7.60
4	8.2	.85	.1037	.60	.0732	10.52
5	7.7	.70	.0909	.60	.0779	5.42
6	11.1	.70	.0631	.65	.0585	22.47
7	8.8	.75	.0852	.60	.0682	9.80
8	7.6	.75	.0987	.60	.0789	11.75
9	9.15	.45	.0491	.45	.0492	10.32
10	6.4	.60	.0937	.50	.0781	2.85
11	5.4	.60	.1111	.50	.0926	2.11
12	6.9	.65	.0942	.60	.0869	4.60
13	5.5	.60	.1090	.30	.0545	2.05
14	8.6	.50	.0581	.50	.0581	5.95
15	6.15	.60	.0975	.50	.0813	3.15

Lot C.

	Length Sp.	Reg Ca.	Sp Reg. Caudal.	Reg. r. Pec	Reg. l. Pec.	Ave. R. L Sp. Reg. Pec	Wt. Sp.
1	11.5	.55	.0478	.55	.55	.0478	23.58
2	9.15	.85	.0929	.45	.45	.0492	12.37
3	9.7	.65	.0670	.50	.45	.0489	11.1
4	7.5	.60	.0800	.60	.60	.0800	5.72
5	7.5	.50	.0666	.35	.40	.0500	4.2
6	10.0	.75	.0750	.70	.70	.0700	14.6
7	6.3	.60	.0952	.50	.55	.0833	4.1
8	6.1	.76	.1065	.40	.50	.0737	3.08
9	5.8	.80	.1379	.45	.40	.0733	2.78

two series do not differ as to that character. If, however, the mean difference is 1 + times the probable difference then it is possible that the two series do differ as to the value of that character. Also if the mean difference is 2 + times the probable difference then it is probable that the two series differ. Finally if the mean difference is 3 + times the probable difference then it is certain that the two series differ as to the value of that character. To apply the law here it is necessary to find the mean of each series, the probable error, and from these compute the mean difference and the probable error of difference.

We can arrange the results as to the caudal fin in Table I. in the form of a table.

Mean Sp. Reg.	Probable Error.	Mean Difference.	Probable Difference.
Lot A = .0860	.003106	Between A and B .0009	Between A and B .004363
Lot B = .0851	.003065	Between B and C .0005	Between B and C .005865
Lot C = .0846	.004991	Between A and C .0014	Between A and C .005876

Comparing A and B we see at once that the mean difference is actually less than the probable difference, and hence this precludes the possibility of any rational conclusion that there is any difference in regeneration in cases A and B. But we have seen that injury in case of A was less than in B. Hence we cannot conclude that the rate of regeneration is greater in the case of the less injured nor in the case of the more injured. The regeneration is the *same*.

We find the same result when we compare B and C, and also when we compare A and C. This experiment tends therefore to negative the results of both Zeleny and Emmel.

We have thus far tested the question by comparing results in these three lots as to regeneration of the caudal fin. But we can also apply the test as to regeneration of the pectorals. We can average the results of the two pectorals in Lot C.

Mean.	Prob. Error.	Mean Diff.	Prob. Diff.
Series B = .0694	.002353		
Series C = .0660	.002752	.0034	.0036

In this case also the mean difference is less than the probable difference hence the two series are the same as to the amount of regeneration in the pectoral fin — although the amount of injury was different in the two cases.

TABLE II.

Lot D.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Wt. Sp.
1	9.9 cm.	.85 cm.	.0858	16.32 gm.
2	9.2	.70	.0760	13.15
3	8.6	.70	.0818	11.35
4	7.2	.80	.1111	11.52
5	8.9	.70	.0786	6.00
6	7.2	.80	.1111	4.09
7	6.4	.65	.1015	7.10
8	7.1	.85	.1197	6.80
9	7.6	.80	.1052	4.33
10	7.9	.75	.0949	7.22
11	6.1	.70	.1147	5.85
12	7.0	.75	.1071	8.30
13	4.5	.60	.1333	1.37

Lot E.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. R. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	8.6	.70	.0818	.60	.0697	11.00
2	10.35	.55	.0531	.60	.0579	16.52
3	7.5	.60	.0800	.40	.0500	6.00
4	7.8	.70	.0897	.60	.0897	8.25
5	8.5	.70	.0823	.50	.0588	11.35
6	7.8	.70	.0897	.55	.0705	8.6
7	8.1	.50	.0617	.50	.0617	9.00
8	10.3	.70	.0679	.70	.0679	17.87
9	8.8	.70	.0795	.50	.0568	10.06
10	6.2	.60	.0967	.40	.0646	4.6
11	8.7	.70	.0804	.50	.0574	13.52
12	6.4	.80	.1250	.50	.0781	5.32
13	8.4	.70	.0833	.60	.0714	10.72
14	6.0	.75	.1250	.50	.0833	3.95
15	7.4	.60	.0811	.50	.0675	8.20
16	6.2	.70	.1129	.55	.0887	4.15

Lot F.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. L. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	7.25	.60	.0827	.60	.0827	5.67
2	7.30	.60	.0821	.35	.0410	8.03
3	7.00	.70	.1000	.50	.0714	6.47
4	6.00	.70	.1166	.60	.1000	4.07
5	6.20	.70	.1129	.40	.0646	4.63
6	6.60	.70	.1060	.60	.1060	5.28
7	6.20	.60	.0967	.50	.0806	3.98
8	6.50	.65	.1000	.40	.0631	5.32
9	6.85	.70	.1022	.60	.0876	3.91
10	6.50	.60	.0923	.45	.0692	5.13
11	6.10	.70	.1147	.50	.0819	4.27
12	6.40	.75	.1172	.35	.1171	4.63

A second experiment was started on August 2 and continued until September 5 a period of four weeks and five days practically the same as those in Table I. In this case the fish were fed regularly for it was desired to test the effect of difference in food on regeneration. In Series D the caudal alone was cut, in E the caudal and right pectoral, in F the caudal and left pectoral. Following is the table of results, Table II.

We may arrange the results as before and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot D	.1010	.002956	Between D and E .0155	.004355
Lot E	.0855	.003199	Between D and F .0018	.003977
Lot F	.1028	.002699		

Since but one pectoral fin was cut off in both E and F it is clear that we cannot compare difference in regeneration in those two lots since the degree of injury was the same. In this case we compare E with D and F with D. Here is the first exception to the uniform results obtained in series A, B and C from Table I. For on comparing E with D we find that the mean difference in regeneration is more than three times the probable difference, hence according to our formula regeneration is really greater in D than in E. At first we might think that since the two differ as to degree of injury that this must be the cause of the result — that is, that in this case at least the least injured regenerated more in the same time. But is this the case? For comparing D and F (the injury to F being the same as that to E) we see that the mean difference between the two is less than the probable difference which shows that no importance can be assigned to that mean difference and that D and F regenerated practically the same amount in the same time. The case of D and F confirms the result already found in A–B, B–C and A–C as to caudal, and in case of B–C as to pectoral. The case of D–F also shows that whatever is the cause of the discrepancy between that and D–E we cannot say that E regenerated less because it was injured more than D.

Table III.

Lot G.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.
1	6.1	.30	.0491
2	6.2	.25	.0403
3	7.5	.15	.0200
4	6.9	.25	.0362
5	5.9	.30	.0508
6	6.5	.30	.0461
7	7.4	.20	.0270
8	8.1	.30	.0370
9	6.5	.20	.0307
10	7.0	.20	.0285
11	7.0	.30	.0428
12	7.0	.30	.0428
13	6.8	.20	.0293

Lot H.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.
1	9.0	.20	.0222	.20	.0222
2	7.2	.40	.0555	.20	.0277
3	6.5	.25	.0384	.25	.0384
4	7.7	.20	.0259	.20	.0259
5	6.2	.30	.0484	.25	.0403
6	6.4	.20	.0312	.20	.0312
7	6.8	.30	.0441	.30	.0441
8	7.1	.30	.0422	.25	.0352
9	7.2	.20	.0277	.10	.0138
10	6.4	.30	.0468	.20	.0312

Lot I.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.	Amt. Reg. Dors.
1	7.3	.30	.0410	.20	.0274	.10
2	6.6	.30	.0454	.25	.0378	.10
3	7.2	.30	.0416	.15	.0208	.10
4	6.5	.25	.0384	.30	.0461	.15
5	7.2	.20	.0277	.20	.0277	.15
6	7.5	.20	.0266	.15	.0200	.10
7	6.4	.30	.0468	.25	.0390	.10
8	7.1	.30	.0422	.25	.0352	.15

In order to test the same question further a third experiment was set up as follows: Three lots of fishes G, H and I were operated on as follows. In G the caudal fin alone was cut off; in H the caudal and one pectoral; in I the caudal, one pectoral and the dorsal. All the fish were placed in a large aquarium with running sea water and were fed regularly. The experiment was begun on September 1 and continued until September 14, 1906. Since all the fish were kept in the same tank there is opportunity for ascertaining whether the discrepancy in case of lot E might not be due to some condition peculiar to the compartment in which they were kept during the five weeks they were under observation. If the results in case of G–H and I confirm the results in cases A–B, A–C, B–C, D–F, etc., then I think we are warranted in concluding that some other condition other than degree of injury was responsible for that discrepancy in case of E. The fishes in this third experiment were fed every day or so. Following is Table III. giving the measurements of these fishes.

Treating these results as before we have the following table :

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot G	.0370	.001483	Between G and H .0005	.002611
Lot H	.0375	.002149	Between H and I .0013	.003539
Lot I	.0388	.002813	Between G and I .0018	.003179

We thus see that in this case also the mean difference is not great enough to ascribe any importance to it. In each case it is less than the probable difference and hence although the series differ as to degree of injury, they do not differ as to rate of regeneration. We can also tabulate the comparative regeneration in the case of the pectorals I and H.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot H	0.0312	.001871		
Lot I	0.0320	.001985	.0008	.002727

In this case also the mean difference is less than three times the probable difference which but corroborates the above contention. We therefore find that in nine cases the result is that although the fishes differed as to degree of injury, the rate of regeneration was the same. The experiments are certainly varied enough to represent different lengths of time, etc. There is but one exception to the results and that we have shown cannot be due to difference in degree of injury.

(C) Relation of Length and Weight (i. e., Age) of Specimen to Amount Rate of Regeneration.

Zeleny ('03) observed that the rate of regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the size of the animal — that the medium sized animals have the maximum rate of regeneration. We quote from his summary, "There is a definite relation between the size (age) of the animal and the rate of regeneration. The maximum rate is exhibited by individuals of medium size. Both the larger and the smaller ones give a diminishing rate as we go away from this point." Zeleny measures size and thus age by the disk width. The three tables given in this paper furnish us with data for determining whether regeneration in *Fundulus* is greater in the smaller, medium, or larger individuals. It occurred to the writer that we ought not only to take length but weight as an indication of age. Unfortunately the specimens in Table III. were not weighed and hence will not be made use of in this comparison. It has been shown by others that regeneration is a phenomenon closely related to growth. Minot has established the fact that growth is greatest in the younger forms. Hence we should expect *a priori* that regeneration should also have a greater rate in the young than in the older forms. For the purposes of this study we can take the regene-

Table IV.

Shortest.			Medium.			Longest.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.
6.5	.60	.0923	8.1	.55	.0676	10.2	.75	.0735
6.35	.80	.1259	8	.65	.0813	11.3	.7	.0610
7.25	.65	.0896	9.2	.65	.0706	11.1	.7	.0631
6.35	.60	.0944	8.25	.8	.0969	11.5	.55	.0478
7	.65	.0915	9.4	.7	.0744	10.0	.75	.0750
6.4	.60	.0937	8.4	.8	.0952			
5.4	.60	.1111	8.4	.6	.0714			
6.9	.65	.0942	8.3	.7	.0843			
5.5	.60	.1090	8.2	.85	.1037			
6.15	.60	.0975	7.7	.7	.0909			
6.30	.60	.0952	8.8	.75	.0852			
6.10	.65	.1065	7.6	.75	.0987			
5.8	.80	.1375	9.15	.45	.0491			
7.5	.60	.0800	8.6	.5	.0581			
7.5	.50	.0666	9.15	.85	.0929			
			9.7	.65	.0670			
			7.9	6.0	.0760			

ration in the caudal fin of all the fishes represented in Table I. This irrespective of the fact that they differ as to degree of injury, since it has just been shown that that has not affected the rate of regeneration. In Table I, we have 37 specimens — the shortest is 5.4 cm. long while the longest is 11.5 cm. long. We can divide the 37 fishes into three lots (1) the shorter, those between 5.4 cm. and 7.5 cm.; the medium between 7.6 cm. and 9.7 cm. while the longer are between 9.8 cm. and 11.9 cm. Arranging all the specimens in these three groups we have the results shown in Table IV.

We find that the average specific regeneration of the shorter is .1028, that of the medium is .0797, while that of the longer is .0638. In other words, the shortest have regenerated 10 + per cent. of their own length, the medium-sized have regenerated 7 + per cent. of their length, while the longest have regenerated 6 + per cent. of their length. Thus the result is contrary to that found by Zeleny with the brittle star-fish. But to make certain we can employ the statistical method used above and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	.0972	.002823	Between S and M =.0190	.003698
Medium	.0782	.002390	Between M and L =.0096	.003786
Longest	.0686	.002934	Between S and L =.0286	.004071

Now we find that the mean difference between S and M is not only more than three times the probable difference but nearly six times as large so that there can be no question but that the shortest have regenerated at a greater rate. On comparing S with L we find that the mean difference in regeneration is over seven times the probable difference. So that our conclusion is that the shorter have regenerated at a much greater rate than either the medium or the longer and that also the medium have regenerated at a greater rate than the longest. Now this last is confirmed when we compare M and L. In this case the mean difference is not quite three times the probable difference but more than twice the probable difference so that it is more than probable that the medium have regenerated more than the longer.

But we also have Table II. for comparison. The shorter specimens in this series are those between 4.5 cm. and 6.45 cm; the medium between 6.46 and 8.41 and the longer between 8.42 and 10.37 cm. Arranging the specimens we have Table V.

TABLE V.

Shorter.			Medium.			Longer.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.
6.4	.65	.1015	7.2	.8	.1111	9.9	.85	.0858
6.1	.7	.1147	7.2	.8	.1111	9.2	.7	.0760
4.5	.6	.1333	7.1	.85	.1197	8.6	.7	.0818
6.2	.6	.0967	7.6	.8	.1052	8.9	.7	.0786
6.4	.88	.1250	7.9	.75	.0949	8.6	.7	.0818
6	.75	.1250	7	.75	.1071	10.35	.55	.0531
6.2	.7	.1129	7.5	.6	.0800	8.5	.7	.0823
6	.7	.1166	7.8	.7	.0897	10.3	.7	.0679
6.2	.7	.1129	7.8	.7	.0897	8.8	.7	.0795
6.2	.6	.0967	8.1	.5	.0617	8.7	.7	.0804
6.1	.7	.1147	8.4	.7	.0843			
6.4	.75	.1172	7.4	.6	.0811			
			7.25	.6	.0827			
			7.3	.6	.0821			
			7	.7	.1000			
			6.6	.7	.1060			
			6.5	.65	.1000			
			6.8	.7	.1022			
			6.5	.6	.0923			

Treating the results as before, we find the average specific regeneration for the shortest is .1136, that of the medium is .0944 while that of the longest is .0763. In other words the shortest of this series of fed fishes have regenerated 11+ per cent. of their length, the medium sized have regenerated 9 + per cent. of their length while the longest have regenerated 7 + per cent. of their length.

In this case also we can apply the statistical method tabulating our results in the following form.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	=.1137	.002897	Between S and M =.0203	.003492
Medium	=.0934	.001949	Between M and L =.0170	.003066
Longest	=.0764	.002367	Between S and L =.0373	.003741

Here again it will be seen that the mean difference in regeneration between S and M is over three times the probable difference, in fact nearly six times; the mean difference between S and L shows that it is over nine times the probable difference so that there can be no doubt that the shortest specimens in Table II. regenerated at a more rapid rate than did the medium or the longest. On comparing the medium with the longest we find that the medium regenerated more rapidly than the longest as is evidenced by the fact that the mean difference is over five times the probable difference.

If we extend our hypothesis that there is a direct relation between growth and regeneration further it would be to find out whether as the fish grew older, the rate of regeneration decreased. For this is precisely the condition as regards growth — the rate of growth decreases with age. At first the decrease is rapid but then becomes very gradual. This is indicated with regard to regeneration. For on comparing regeneration of the shortest with the medium and the regeneration in the medium with the longest we discover an indication of this relation. In each of the two cases it is seen that mean difference in regeneration is greater between shortest and medium as compared with the mean difference in regeneration between the medium and longest.

It was said at the beginning of this section that in estimating age we ought to consider both length and weight. So I have placed opposite the length, etc., of each specimen its weight. *A priori*, the youngest ought to be the shortest and lightest, etc. This is the case here, for the average weight of the shortest is 3.38 gm.; the average weight of the medium length is 8.96 gm., while that of the longest is 20.85 gm. So that in Table IV. our three classes represent the youngest, the oldest and those midway between the others in age. In the same way for Table V. the average weight of the shortest is 4.48 gm.; that of the longest is 12.71 gm. while that of the medium length is 7.52 gm. In this case also our three series in Table V. represent the youngest, the oldest and the medium aged fishes of that lot. We conclude therefore that the rate of regeneration in *Fundulus h.* is greatest in the youngest fishes, less in the older, and that there is a slight indication that the rate of regeneration decreases with age.

(D) Relation of Regeneration to Food.

It will be remembered that fishes in Series A, B and C were not fed while those in Series D, E and F were fed. Otherwise as far as known they were kept under identical conditions and for the same length of time. It was desired to compare regeneration in the two series with each other to ascertain whether regeneration is at a greater rate in the fed fishes than in the not-fed. Unfortunately we cannot make use of Series E because as before said some factor has entered there and caused a result which I do not believe to be normal. On the other hand I do regard Series D and F as normal. Therefore we must compare regeneration in D with that in A.

I. Caudal.	Mean Reg.	Prob. Error.	Mean Diff	Prob. Diff.
Lot D (fed)	—.1010	.003106		
Lot A (not fed)	=.0860	.002958	.0150	.004249

In this case the mean difference in regeneration is more than three times the probable difference. In other words the fishes in Lot D which were fed have regenerated more in the same time than the fishes in Lot A which were not fed.

II. Caudal.	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot F (fed)	.1028	.002659		
Lot B (not fed)	=.0851	.003065	.0177	.004084

In this case also the mean difference in regeneration between F and B is more than three times the probable difference. Or the fishes in Lot F which were fed have regenerated more than the fishes in Lot B which were not fed.

We may compare the average specific regeneration of the caudal in the case of the fishes that were fed with that in the fishes that were not fed in the following way also.

Average Sp. Reg. in caudal of Lots D + F (fed)............................ = 0.10100
Average Sp. Reg. in caudal of Lots A + B + C (not fed).................. = 0.08565
Difference.. = 0.01535

The caudal fin in fishes that were fed regenerated 18 per cent. more than that in fishes not fed.

Again,

Average Sp. Reg. in pectoral fin in Lot F (fed)................................ = 0.0866
Average Sp. Reg. in pectoral fin in Lot B + C (not fed)...................... = 0.0677
Difference.. 0.0189

that is, the pectoral in the fed fishes regenerated 24 per cent. more than that in the unfed.

(E) Regeneration in Fins used most Compared with that in Less used Fins.

Adaptation in Regeneration. — Osburn ('06) experimenting on the fins in *Fundulus* finds that the pectorals are not used as vigorously as the caudal, and the dorsal not so much as the pectorals. Broussonet (1786) stated that the most useful fins regenerate more rapidly than those less useful. Morrill ('06) says that the "caudal fin though obviously the most important does not regenerate perceptibly faster than the others." Although the data are insufficient to settle this question which is to a great extent a matter of interpretation yet the following table is suggestive. Since it has been shown that degree of injury has made no difference in the rate of regeneration in *Fundulus h.*, it is therefore possible to group all the specimens represented in Table I. thus,

Average Sp. Reg. of caudal fin in Lots A + B + C	= 0.08565
Average Sp. Reg. of pectoral fin in Lots B + C	= 0.06770
Difference	= 0.01795

which means that the caudal fin of specimens in Lots A + B + C regenerated 26 per cent. more than the pectoral fin in Lots B + C in the same time and under same conditions.

In the same way from Table II., we find

Average Sp. Reg. of caudal fin in Lots D + F	= 0.01016
Average Sp. Reg. of pectoral fin in Lot F	= 0.00846
Difference	= 0.00170

or in other words the caudal regenerated 20 per cent. more than the pectoral.

Again in Table III. the

Average Sp. Reg. in caudal in Lots G + H + I	= 0.0386
Average Sp. Reg. in pectoral in Lots H + I	= 0.0313
Difference	= 0.0073

or the caudal in this case has regenerated 20 per cent. more than the pectoral. Thus in all three cases the result is the same. It should be remembered that specific regeneration is the relation of amount regenerated to the length of the specimen. And hence we have a right to compare the specific regenerations of caudal with pectoral. It may also be pointed out here that the dorsal fin regenerates very slowly as compared with the other two as may be seen in Table III.

GENERAL CONCLUSIONS.

1. That low temperatures inhibit regeneration in caudal fin of *Fundulus heteroclitus.*

2. That the rate of regeneration bears no relation to the degree of injury to the caudal and pectoral fins.

3. That regeneration is greater in the younger than in the medium and older fishes which is in line with the theory that regeneration is a growth phenomenon.

4. That regeneration is greater in the fish that have been fed as compared with that in the fishes not fed.

5. That there *is* an indication that the fins used or needed most (*i. e.*, caudal) regenerate more rapidly than the less needed fin (pectoral), or in other words there is an indication of adaptation in regeneration.

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